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Abstract Book

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Oral Presentation Abstracts

Medical Microbiology
Host-pathogen Interactions

Session Leads

Amy Tvinnereim, PhD (UTHSCT)

Buka Samten, MD (UTHSCT)

Estrogen Signaling Contributes to Group B Streptococcal Interaction of the Blood Brain Barrier

Kathryn Seely[1], Nadine Vollmuth[2], Vishwas Subash[1], Brandon Kim[2]

[1]Department of Biological Sciences, The University of Texas at Dallas, Richardson, United States. [2]Department of Biological Sciences, University of Alabama, Tuscaloosa, United States

Abstract

Group B Streptococcus (GBS) is a pathobiont and the leading cause of neonatal meningitis. Previous studies have demonstrated a link between maternal perinatal hormone treatment and increased risk of neonatal GBS infection. To investigate the role of estrogen signaling in this process, induced pluripotent stem cell–derived brain-like endothelial cells (iBECs) [ks1] were treated with β-estradiol (E2) prior to GBS infection. These experiments revealed an increase in bacterial invasion of the blood–brain barrier (BBB). We hypothesize that the E2 hormone not only impacts the BBB directly, but also may modulate the expression of GBS virulence factors. To test this hypothesis, cultures of GBS were exposed to controlled concentrations of E2, and total RNA was extracted for transcriptomic analysis. RNA quality and purity were assessed prior to sequencing. Quantitative PCR was performed to evaluate expression of key virulence-associated genes, focusing on *srr* and *pili* to begin exploring the bacterial response to host sex hormones. By qPCR we observe an upregulation of the adhesin-encoding *pili* gene. RNA-seq is being conducted and is expected to provide a complementary view of global gene regulation that may reveal a microbial response to host hormones. Future studies will investigate the effect of progesterone on the GBS transcriptome using qPCR and evaluate deferentially regulated genes in adherence and invasion assays with the BBB. The results of this study will advance understanding of how sex hormone signaling influences GBS pathogenesis, informing future strategies to mitigate neonatal infection.

Evolutionary Landscape of Colistin Resistance in *Acinetobacter baumannii*

Muneer Yaqub

The University of Texas at Dallas, Richardson, United States

Abstract

Nosocomial infections caused by *Acinetobacter baumannii* are an escalating public health challenge due to this pathogen's rising resistance to antibiotics, particularly polymyxin E (colistin), a critical last-line therapy. Because colistin resistance rates remain relatively low in *A. baumannii*, its underlying mechanisms are still poorly understood. To address this gap, we applied a machine learning model trained on clinical isolates, identifying 31 candidate genes linked to colistin resistance. These genes encode diverse functions, including membrane biosynthesis, iron transport, transcriptional regulation, and DNA repair. Using the Manoil AB5075 transposon library, we evaluated mutant phenotypes through antimicrobial susceptibility testing and growth kinetics under sub-inhibitory colistin exposure. While MIC values were unchanged relative to the parent strain, nearly 68% of the mutants exhibited reduced fitness and 13% displayed enhanced fitness under sublethal colistin stress, accompanied by alterations in membrane properties, biofilm production, efflux pump activity, and oxidative stress response. To further test the in vivo relevance of these findings, we employed a murine pneumonia model. Several mutants demonstrated impaired survival in the lung environment, underscoring the importance of adaptive fitness mechanisms beyond MIC-defined resistance. Collectively, our results highlight the limitations of standard susceptibility assays in capturing the full spectrum of colistin resistance and reveal how genetic determinants shape bacterial persistence in both in vitro and in vivo contexts. These insights emphasize the need to redefine how resistance is measured in *A. baumannii*, with implications for developing more accurate diagnostics and improved therapeutic strategies.

Paradoxical Effects of *Ascaris*-Induced Pyloric Metaplasia: Reducing *Ascaris* Reinfection Burden but Creating *Helicobacter pylori* Opportunity

Nina Tang[1], Yifan Wu[1], Charlie Suarez-Reyes[1], Alexander Kneubehl[1], Jill Weatherhead[1,2]

[1]Baylor College of Medicine, Houston, United States. [2]National School of Tropical Medicine, Houston, United States

Abstract

Ascaris roundworms infect over 700 million individuals globally and disproportionately affect low- and middle-income countries (LMICs). Reinfection in LMICs is common with an age-dependent phenomenon wherein children harbor heavier infectious burdens than adults. Given our lab's discovery that *Ascaris* hatching and larval migration occurs in the highly plastic environment of the stomach, we hypothesized that age-dependent variability in *Ascaris* infectious burden was due to gastric mucosal reprogramming following early infectious insults. Using a novel murine model of repeated *Ascaris* infection, we found that repeated *Ascaris* challenge lowered infectious burdens in BALB/c mice compared to single *Ascaris* challenge, indicating adaptive protection against heavy infection. This protection was due to *Ascaris*-induced pyloric metaplasia (AIPM) – a reparative phenotype characterized by parietal cell death, reduction of gastric acid production, and chief cell transdifferentiation – not adaptive immunity. Although protective against heavy parasitic burdens, we also found that AIPM increases expression of surface proteins used by the gastric bacterium, *Helicobacter pylori,* for adherence to the gastric mucosa. *H. pylori* is a primary driver of chronic gastric disease and is especially prevalent in LMICs where it shares significant geographic overlap with *Ascaris* and gastric cancer incidence is elevated. Our data on AIPM provides novel insights on the reinfection dynamics of *Ascaris* and suggests a previously unexplored link between *Ascaris* and *H. pylori*. This mechanism for *Ascaris* infection increasing host susceptibility to *H. pylori* colonization may yield a new – highly treatable – infectious etiology of gastric cancer.

Mast Cell-Specific G Protein-Coupled Receptor Regulates Bladder Immunity During Urinary Tract Infections

Waris Muhammad Khuwaja[1], Colin Guth[2], Linghui Nie[3], Nicole De Nisco[1], Priyanka Pundir[2], Dustin Green[3], Xintong Dong[1]

[1]University of Texas at Dallas, Richardson, United States. [2]University of Guelph, Guelph, Canada. [3]University of Texas Medical Branch, Galveston, United States

Abstract

Urinary tract infections (UTIs), predominantly caused by uropathogenic *Escherichia coli* (UPEC), affect millions of women each year. During infection, the bladder initiates an immune response by recruiting various immune cells to eliminate the pathogen. Mast cells, tissue-resident granulocytes primarily known for their roles in type 2 immunity and allergic responses, also function as sentinels against invading microbes. The mast cell-specific G protein-coupled receptor (GPCR) Mrgprb2, the murine homolog of human MRGPRX2, has been shown to detect bacterial quorum sensing peptides and facilitate neutrophil recruitment in skin, lung, and peritoneal infections. However, its role in bladder immunity remains unclear. To investigate the function of Mrgprb2 in UTIs, we infected 7-10-weeks-old female C57BL/6J wild-type (WT) and *Mrgprb2*-knockout (KO) mice with 10^7 CFUs of UPEC strain UTI89 for 24 hours. Bladders were collected for CFU enumeration, bulk RNA sequencing, flow cytometry, and histological analysis. Surprisingly, *Mrgprb2*-KO mice exhibited enhanced bacterial clearance compared to WT controls. RNA-seq analysis revealed that Mrgprb2 regulates key aspects of bladder immune responses, including immune cell recruitment, urothelial exfoliation, and cell proliferation. Flow cytometry showed reduced immune cell infiltration and inflammation in KO mice post-infection, suggesting that excessive inflammation may promote UPEC colonization. Histological analysis further indicated increased epithelial proliferation and improved tissue repair in KO mice. We are currently investigating if the Mrgprb2 ligand cathelicidin, an antimicrobial peptide, mediates these immunomodulatory effects. Our findings identify a previously unrecognized role for mast cells and Mrgprb2/MRGPRX2 signaling in bladder immunity, presenting them as potential therapeutic targets to improve UTI outcomes.

In Vivo Characterization of Microbe Host Interaction Between *Staphylococcus aureus* and the Mammalian Defensin System

Bhavani Balasundarasekar[1], Matthew Thomas[1], Monica Thanigaivel[1], Rebecca Keogh[2], Jacob Hogins[1], Alexander Horswill[2], Xintong Dong[1]

[1]The University of Texas at Dallas, Richardson, United States. [2]University of Colorado Anschutz Medical Campus, Aurora, United States

Abstract

Staphylococcus aureus (SA) is responsible for a wide range of skin infections, from superficial impetigo to deep abscesses, and the rise of methicillin-resistant strains (MRSA) has made it a major public health concern. The skin's innate defenses rely heavily on antimicrobial peptides (AMPs) such as defensins, which not only kill bacteria but also recruit neutrophils through the Mrgpra2 receptor. When defensins are missing, as in defensin cluster knockout (Def-cKO) mice, immunity is impaired, bacterial burden rises, and neutrophil recruitment is reduced, but how SA adapts to this pressure has remained unclear. To explore this, we used RNAseq to profile SA recovered from WT, Def-cKO, Mrgpra2-deficient, and neutrophil-ablated mice and found that within 24 hours the bacteria undergo sweeping transcriptional reprogramming, upregulating toxins and secreted virulence factors while reducing surface adhesins. Two genes, *dltA* and *ssaA*, stood out as strongly induced. Functional infection studies showed that while Δ*dltA* and Δ*ssaA* mutants persisted at levels similar to WT bacteria in WT mice, both were cleared more efficiently in Def-cKO mice. This counterintuitive result indicates that defensins create a selective environment where *dltA* and *ssaA* provide survival benefits, highlighting the active role of AMPs in shaping pathogen fitness. Our work shows that defensins actively shape how *SA* adapts in the skin and that genes like *dltA* and *ssaA* are central to this process. By uncovering how these pathways give the bacteria a survival edge, we point to AMP resistance as a vulnerable point that could be exploited for new strategies against MRSA infections.

Recovery of Paracellular Barrier in Pulmonary Epithelial Monolayers Exposed to Pneumolysin from *Streptococcus pneumoniae*

Feranmi Obe, Ali Azghani

University of Texas at Tyler, Tyler, United States

Abstract

Pneumolysin (Ply), a cholesterol-dependent pore-forming toxin produced by *Streptococcus pneumoniae*, compromises epithelial barrier integrity and facilitates dissemination of infection. We hypothesized that lung epithelial cells possess intrinsic repair mechanisms involving tight junction protein expression and redistribution and actin reorganization. Confluent bronchial epithelial monolayers (Calu-3) were exposed to 5 μg/mL of Ply. Transepithelial electrical resistance (TEER) was used to monitor the monolayer's barrier integrity. Tight junction proteins localization and actin remodeling were examined with a confocal microscope and Western blot was used to quantify protein expression. We found that Ply induced a significant drop in TEER within 30 minutes, with transient recovery at 5 hours and subsequent decline at 10 hours. Replacing Ply with serum-free medium at 5 hours led to progressive TEER restoration over 24 hours. Immunofluorescence analysis at recovery time points revealed intracellular redistribution of Occludin and ZO-1, and stress fiber formation at 5-10 hours, and partial cortical actin reorganization at 24 hours. Western blot confirmed decreased Occludin expression within 5 hours of Ply exposure, with gradual restoration at 24 hours post-toxin removal. Ply also induced ERK1/2 phosphorylation at 30 minutes, which further declined, an effect that was attenuated by MEK1/2 inhibitor, PD98059. Pretreatment with PD98059 before Ply exposure partially restored Occludin expression, while PD98059 alone maintained the Occludin at control levels. These findings suggest that Ply-induced barrier disruption involves MAPK/ERK signaling; however, epithelial cells retain the capacity for partial recovery, highlighting potential therapeutic avenues for early mitigation of pneumococcal lung injury.

General Microbiology
Environmental

Session Leads

Riqing Yu, PhD (UTT)

Amanda Morris, PhD (UTHSCT)

Characterizing the Expression and Functions of MRGPR Receptors in Human Neutrophil Cell Line HL-60

Yining Liu, Sharan Kumar, Xintong Dong

University of Texas at Dallas, Richardson, United States

Abstract

The family of Mas-related G protein-coupled receptors (MRGPRs) are itch and pain receptors responsible for sending downstream signaling pathways within a cell. They play a crucial part in initiating inflammation and contribute to diseases such as psoriasis. One of the most prominent immune cells, neutrophils, serves as a key component in initiating the acute phases of inflammation. Mouse neutrophils have expressed Mrgpra2a and Mrgpra2b, but it's unclear which MRGPR receptor is expressed in human neutrophils. There are 8 MRGPR receptors, MRGPRX1, X2, X3, X4, D, E F and G. My project aims to characterize and determine the function of the MRGPRX receptors on human Neutrophils. We first differentiate HL-60 cells into neutrophils. Successful differentiation is tested by assays to validate known neutrophil genes. We then use qPCR to determine which MRGPRX genes are expressed and upregulated in neutrophils. In the future, we will remove *MRGPRX* genes from neutrophils to provide insight to the functionality of the receptors. The findings of this research would allow for a clearer understanding of areas to target when developing drugs and medications to treat diseases such as psoriasis.

Rapid Detection of Shiga Toxin-Producing *E. coli* with MALDI-TOF MS

Maddie Joseph, Madison Prince, Christopher Howard, Michael LaMontagne

University of Houston-Clear Lake, Houston, United States

Abstract

Shiga toxin-producing *E. coli* (STEC), are major food pathogens. STEC strains are also often detected in recreational waters and runoff from agricultural systems. Current methods of identifying STEC include immunoassays, whole genome sequencing and multiplex PCR. These approaches are expensive, time-consuming and require highly trained personnel. Matrix assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) provides a low-cost, rapid method of identifying microbes and is widely used in clinical laboratories; however, few studies have attempted to identify STEC with MALDI-TOF MS. Here, fecal coliforms were isolated from animal and human sources on Eosin Methylene Blue agar and presumptive *E. coli* colonies were re-streaked identified with MALDI -TOF MS. STEC strain O103:H11 was included as a positive control. The program GPMsDB-tk was used to annotate peaks in the mass spectra and to assign taxonomy to the isolates. The isolates were also screened for genes associated with STEC by multiplex PCR. Cluster analysis of mass spectra showed a clade that contained O103:H11 (the STEC reference strain). All other strains in this cluster contained at least one of the genes (*stx1* (~180bp), *stx2* (~255bp), *eaeA* (~384 bp), and *hlyA* (~534 bp)) associated with STEC as assessed by multiplex-PCR. Mass spectra peak annotation identified acid stress proteins (HdeA/HdeB) and major outer membrane lipoprotein Lpp peaks as potential biomarkers for STEC. Together, these results suggest that MALDI-TOF MS shows promise as a rapid method of identifying STEC.

Phthalate-Based Plasticizer Pollutants Modulate Kaposi's Sarcoma-Associated Herpesvirus Infection

Spandan Mukherjee, Erica Sanchez

University of Texas at Dallas, Richardson, United States

Abstract

Kaposi's Sarcoma-associated Herpesvirus (KSHV) is an oncogenic virus that causes Kaposi's Sarcoma (KS) with no known cure. KSHV has stages: latency (low gene expression, no virions) and lytic replication (full gene expression and virion production), which may be affected by environmental pollution. Plasticizers, common plastic additives, are often phthalic acid derivatives, phthalate plasticizers (PPs). Evidence shows that exposure to PPs, di(2-ethylhexyl) phthalate (DEHP), its metabolite Mono-(2-ethylhexyl) phthalate (MEHP), and Diisononyl phthalate (DINP), may increase viral infectivity and have oncogenic potential. We hypothesize that PPs alter gene expression and virus production in KSHV-infected cells. To test our hypothesis, we measured KSHV viral gene expression and viral production in the presence or absence of PP treatment. We utilized iSLK.BAC16 cells, which maintain the KSHV genome, express GFP, and can be induced to lytic replication upon doxycycline (Dox) treatment. iSLK.BAC16 cells were pre-treated with PPs (DEHP, MEHP, or DINP) for 24hrs, then induced to lytic replication for 48hrs. We measured expression of an early (ORF59) and late lytic gene (K8.1) via RT-qPCR and collected supernatant for viral titers. These assays characterize viral replication at each stage. Preliminary data show DEHP significantly decreases late lytic gene expression and reduces infectious virion production. DINP and MEHP decreased viral production, with gene expression being further investigated. This suggests PPs suppress KSHV DNA replication, inhibiting viral replication. Current experiments focus on the mechanism behind PPs inhibiting infection. This work highlights PP exposure as an environmental toxicology concern for virus-host modulation and may reveal therapeutic targets.

Comparative Systems Analysis of Wild-Type and Lysogens of *Streptomyces griseus*: Regulation and Reprogramming of Primary and Secondary Metabolism

Ahmad Sulaiman, Lee Hughes

University of North Texas, Denton, United States

Abstract

Lysogeny, the integration of bacteriophage DNA into a host genome, can profoundly influence bacterial physiology, yet its effects on metabolic regulation in *Streptomyces griseus* remain underexplored. In this study, we identified multiple phages capable of integrating into *S. griseus* genomes and confirmed lysogeny through multiple approaches, while also assessing their antimicrobial activities. Comparative metabolomic profiling was conducted for the wild-type strain and two potential lysogens (*S. griseus* Animus lysogen and *S. griseus* Broplease lysogen). The analysis revealed clear alterations in both central carbon metabolism and secondary metabolite production, including notable shifts in streptomycin biosynthesis. These results demonstrate that lysogeny exerts measurable and distinct effects on metabolic regulation in *S. griseus*. Furthermore, antimicrobial activity assays against alternative ESKAPE-associated bacteria demonstrated that BroPlease, in particular, had enhanced inhibitory effects against S*. epidermidis, K. aerogenes,* and *E. coli*. These findings support the hypothesis that prophage integration not only alters internal metabolic flux but can also expand or reshape the antimicrobial spectrum of *S. griseus*. The next phase of this work will expand metabolomic analysis to an additional lysogen and employ RNA sequencing across all strains to link metabolic changes with underlying transcriptional reprogramming. Together, this integrated systems-level approach will provide new insights into phage-host interactions, their impact on central and secondary metabolism, and potential strategies for optimizing antibiotic production in *S. griseus*.

Beyond the Living: Dead Cells Promote Survival of Live Cells Under Antibiotic Attack

Ha Do, Souvik Bhattacharyya

UTHealth Houston, Houston, United States

Abstract

Antibiotic resistance, the ‘silent pandemic’, is a global health concern. Bacteria employ many strategies to survive antibiotic challenges. They can be genomically resistant via mutation or resistance markers but also can exhibit temporary behavior towards antibiotic survival, e.g., tolerance, persistence, etc. As a natural consequence, many bacteria within a population die in the presence of antibiotics. Recently, we showed that dead cells release a danger signal, called the ‘necrosignal’, promoting antibiotic survival pathways in *E. coli* swarms, a flagella-driven surface motility. However, we do not know whether death impacts live cells in broth culture. Here we show that dead cell extract (DE) promotes survival of live cells at sub-inhibitory concentration of two antibiotics tested, ciprofloxacin and cefiderocol. We found DE promotes tolerance behavior in live cells which was also dependent on the major efflux pump outer membrane protein TolC. Our results suggest how death can be sensed as a danger signal within the population experiencing antibiotic stress. Our preliminary data suggests the involvement of a metabolite or other small molecules in this phenotype. In future, we plan to identify the signal and decipher mechanistic details of this phenomenon. This study will inform on general danger sensing behaviors in bacteria, potentially with clinical implications.

Microbiome Composition and Community Structure in Soil Sediments of Lake Raven and Lake Conroe

Chito Ofodum, Madhusudan Choudhary

Sam Houston State University, Huntsville, United States

Abstract

Freshwater microbes play vital roles in lake ecology and are indicators of natural and human-induced stress due to their rapid response to environmental changes and contribute to maintaining the stability of the structure and function of aquatic ecosystems. Lake Conroe is the reserve drinking water supply for the city of Houston and offers a large variety of recreational resources while Lake Raven is a 203-acre reservoir located in Huntsville State Park and is also used for a variety of recreational activities. The objective of the study is to compare the microbiome composition and diversity between the two lakes. A total of 36 soil sediment samples were collected from the two lakes and stored at -80˚C. Bacterial DNAs were extracted from these soil samples and V3-V4 regions of the 16S rRNA gene were amplified by polymerase chain reaction. Metagenomic sequence library was prepared, and the DNAs were subsequently sequenced using Illumina Mi-Seq. Analysis was performed on QIIME 2 platform, and taxonomic classification was assigned using RDP (Ribosomal Database Project classifier). Results revealed that the microbial community structures at the two study sites share some common genera/species, but they exhibit significant differences at the genus/species level. The observed microbial diversity seems to be associated with metabolic specializations, including nitrification, denitrification, and arsenic detoxification. Future work would closely examine differences of the microbiome structure and function and its involvement in bioremediation processes.

Medical Microbiology
Viruses, Public Health, Medical Microbiology

Session Leads

Michelle Crum, PhD (PHLET)

Guohua Yi, PhD (UTHSCT)

Understanding Infection Initiation in *Burkholderia cenocepacia* phage BcepIL02, a *Lessievirus* Encoding Multiple Tail Fibers and gp64, a Novel Host Range Determinant

Andrej Momiroski, Allyson Sokolowski, Carlos Gonzalez, Jason Gill

Texas A&M University, College Station, United States

Abstract

The *Burkholderia cepacia* complex (Bcc) is a group of Gram-negative bacteria with the ability to colonize and cause severe infection in the lungs of cystic fibrosis (CF) and chronic granulomatous disease (CGD) patients. Bacteriophage therapy is being actively evaluated as an adjunctive treatment to antibiotics for these individuals. *Burkholderia cenocepacia* podophage BcepIL02 (genus *Lessievirus*) contains four predicted tail fiber (TF) genes, all of which are virion-associated. To better understand how BcepIL02 initiates infection in *B. cenocepacia* strain PC184, five phage-insensitive mutants of PC184 were isolated and sequenced, and four of these mutants were found to have mutations in a putative glycosyltransferase associated with LPS biosynthesis. Phage mutants able to overcome host resistance were isolated, with 105 rare plaques that appeared on three of the insensitive strains being isolated and subjected to targeted amplicon sequencing. Only five mutants had mutations in the first TF gene; the other 100 sequenced phages had mutations in one of two genes directly downstream of the TF genes. One gene encodes a putative tail nozzle protein, and the other (gene 64) encodes a short (<150 amino acid) virion-associated protein of unknown function (gp64). Tandem mass spectrometry analysis on three representative mutants found that representatives with either nozzle or gene 64 mutations had gp64 missing from their virions; furthermore, all three representatives had TFs 3 and 4 missing. Together, these results suggest an atypical mechanism for host infection in this phage, involving multiple tail fibers, and other tail proteins.

Evaluating Bacteriophage Therapy Against *Klebsiella pneumoniae* in Innate Immune Cells

Mounika Duggineni[1], Rajesh Mani[1], SitaRamaRaju Adduri[1], Aran Belani[2], Andy Omeje[1], John Dennehy[3], Guohua Yi[1]

[1]The University of Texas Health Science Center at Tyler, Tyler, United States. [2]University of Texas at Austin, Austin, United States. [3]Queens College of The City University of New York, New York, United States

Abstract

Klebsiella pneumoniae is a major cause of multidrug-resistant infections, posing a critical threat to global health. This study aimed to isolate and characterize broad-host-range bacteriophages with therapeutic potential against diverse clinical *K. pneumoniae* strains and evaluate their efficacy in vitro, ex vivo, and in vivo. Clinical *K. pneumoniae* strains and phages were isolated from hospital wastewater, propagated, and quantified using standard plaque assays. Seven bacterial and fifteen lytic phage strains, representing genera such as *Marfavirus*, *Slopekvirus*, *Webervirus*, and *Jiaodavirus*, were successfully isolated. Among purification methods, ultrafiltration yielded higher titers of functional phages compared to polyethylene glycol-based purification. Serial dilution plaque assays identified three high-titer phage isolates for further testing. In vitro killing assays, performed by co-incubating bacteria and phages and measuring OD600, showed up to a 13-fold reduction in bacterial load within four hours. Encouraged by these results, we assessed phage activity in primary human immune cells such as macrophages and neutrophils, derived from peripheral blood mononuclear cells of healthy donors. Upon infecting primary cells with *K. pneumoniae* and treating with phages, we observed bacterial colony forming units (CFU) reductions of 19,000–71,000-fold and 15,000–33,000-fold at multiplicities of infection (MOI) of 10 and 20, respectively. Altogether, the phage isolates demonstrated robust efficacy against *K. pneumoniae*, providing a promising preclinical foundation for phage therapy as an alternative to antibiotics. Future studies will evaluate the efficacy of phage cocktails in a humanized NSG-SGM3-IL15 mouse model of *K. pneumoniae* infection.

Developing an Immunogenic and Cross-Protective Flavivirus mRNA Vaccine

Maxwell Ross[1], Megan Carey[2], Allyson Hildago[3], Daniel Rehm[4], Jeroen Pollet[5], Athos Silva de Oliviera[6], Jennifer Clinton[7]

[1]Graduate School of Biomedical Sciences at Baylor College of Medicine, Houston, United States. [2]Department of Pediatrics, Division of Tropical Medicine, Baylor College of Medicine and Texas Children's Hospital, Houston, United States. [3]Department of Pediatrics, Division of Neurology and Developmental Neuroscience, Baylor College of Medicine and Texas Children's Hospital, Houston, United States. [4]William T. Shearer Center for Human Immunobiology, Texas Children's Hospital, Houston, United States. [5]Department of Medicine, Section of Immunology, Allergy, and Rheumatology, Baylor College of Medicine, Houston, United States. [6]Center for Vaccine Development, Texas Children's Hospital, Houston, United States. [7]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, United States

Abstract

Zika (ZIKV), West Nile (WNV), and dengue are positive-stranded, RNA flaviviruses transmitted by arthropod vectors including *Aedes* and *Culex* mosquitoes. Collectively, flaviviruses cause >400 million acute infections and >25,000 deaths annually. Clinical disease ranges from flu-like symptoms to severe neuroinvasive (encephalitis, meningitis), hemorrhagic, or congenital (microcephaly, miscarriage, stillbirths) outcomes. Licensed vaccines remain unavailable for most flaviviruses, including ZIKV and WNV, leaving supportive care as the only option. This project seeks to develop an mRNA vaccine that elicits both humoral and cellular immunity and confers cross-protection against multiple flaviviruses. Our candidates target the ZIKV envelope (Env) protein, a highly conserved antigen, to enhance immune cross-protection. Five ZIKV Env mRNA constructs containing different signal sequences were generated and formulated in lipid nanoparticles. Protein expression was confirmed *in vitro* by Western blots and immunofluorescence. Immunogenicity was evaluated in C57BL/6 mice (n=30) using a prime-boost regimen, with sera and spleens collected three weeks post-boost. Flow cytometry of splenocytes revealed that Env vaccinated mice mounted significantly higher antigen-specific $CD4^+$, $CD8^+$, and INF-y-producing $CD8^+$ responses compared to controls. These data demonstrate vaccine-induced T-cell activation and cytokine expression, highlighting the vaccine's ability to elicit cell-mediated immunity. We are currently quantifying total and neutralizing antibody titers via ELISA and plaque reduction neutralization tests. Future studies will evaluate efficacy in murine viral challenge models. As climate change drives mosquito expansion into new regions, a cross-protective vaccine would help mitigate emerging outbreaks, protect vulnerable populations, and reduce the global burden of multiple flavivirus infections.

Engineering HIV-1 Envelope (Env) Glycoprotein Using Genetic Codon Expansion to Gain Insights into Structural Dynamics of Env

Narendra Kumar Gonepudi, Wang Xu, Revan siddha H Katte, Maolin Lu

University of Texas at Tyler, Tyler, United States

Abstract

The HIV-1 Envelope (Env) glycoprotein trimers undergo structural changes upon interacting with host receptor CD4 and coreceptors (CCR5/CXCR4) for virus entry. As the only surface-exposed viral protein, Env is a primary target for antibodies but evades recognition through structural rearrangements. Studying Env dynamics with respect to the coreceptor binding region (V3 loop) provides insights into virus entry and immune evasion. Fluorescence labeling via genetic code expansion (amber–TAG suppression) offers a minimal invasive and site-specific approach for fluorescence microscope-based Env dynamics studies. Considering the critical role of V3 loop in coreceptor interactions and immune evasion, along with the novel labeling strategy, our study generated two V3-related dual-amber Env constructs ($A135_{TAG}$-$P308_{TAG}$, $P308_{TAG}$-$E395_{TAG}$) by incorporating two unnatural amino acids at consecutive amber codons in Env on virions. To ensure their biological and functional integrity, a series of assays were performed, infectivity assays and immunoblotting demonstrated ~10-15% amber suppression efficiency, confirming successful Env expression, and incorporation onto virions. Neutralization assays revealed that dual-amber Env-containing virions retained neutralization sensitivities similar to those of wildtype Env-containing virions. Nanoparticle tracking analysis revealed the size distribution of dual amber Env containing viral particles was consistent with that of wild-type virions. Altogether, our study highlights the feasibility of unnatural amino acid incorporation into HIV-1 Env, while maintaining key functional properties. These findings pave a way for advanced click labeling strategies to investigate time-correlated structural dynamics of Env from the V3 perspective, advancing understanding of HIV-1 entry mechanisms and informing vaccine design.

Crimean-Congo Hemorrhagic Fever Virus Infection in Human and Animal Cell Lines: A Pathway to Understanding Species-Specific Pathogenesis

Kiyah Costin

University of Texas Medical Branch, Galveston, United States

Abstract

Crimean-Congo hemorrhagic fever virus (CCHFV) is a zoonotic, tick-borne virus of the *Nairoviridae* family that causes severe hemorrhagic fever in humans. In contrast, infected amplifying hosts such as ruminants and small mammals typically remain asymptomatic. The immunological mechanisms that drive this species-specific pathogenesis remain poorly understood. We hypothesize that bovine cell lines will exhibit lower viral titers and viral replication efficiency, potentially due to unidentified host-specific viral evasion factors. We are utilizing immunofluorescence (IFA) and plaque assays using SW13 (human) and MDBK (bovine) cell lines, with Hazara virus (HAZV) serving as a BSL-2 surrogate for CCHFV, to investigate potential differences in viral entry and replication between human and animal hosts. Additional assays, including viral growth kinetics and focus-forming assays, will be conducted to further evaluate species-specific viral dynamics. Findings from this research will aid in identifying host factors that contribute to species-specific pathogenesis during CCHFV infection.

Cellular Targets and Pathogenesis of Bourbon Virus at the Tick-Host-Virus Interface

Megan Burch, Catherine Olal, Dennis Bente

University of Texas Medical Branch, Galveston, United States

Abstract

Bourbon virus (BRBV) is an emerging tick-borne virus in the Midwest and southern United States, with five confirmed human cases and two fatalities. The vector for BRBV, *Amblyomma americanum,* also known as the lone star tick, is rapidly expanding geographically, increasing the risk of BRBV spreading further in the US. Currently, the pathogenesis of BRBV, including early cellular targets, remains poorly understood. Our goal is to define the kinetics of infection and identify cellular targets at the tick-host-virus interface. To this end, we have developed an *ex vivo* skin model that can be used to investigate BRBV tropism in the skin. Human skin discarded from abdominoplasty surgery was used to generate *ex vivo* skin explants for infection via intradermal injection or abrasion with topical application. Explants were assessed for infection and viral shedding with plaque assays, pathogenic changes in the skin structure with H&E staining, and infected cell types with immunohistochemistry. Initial results show that BRBV infects and replicates in the *ex vivo* model and causes pathological changes to the skin. Future studies will focus on the influence of tick saliva on BRBV pathogenesis, in-depth characterization of infected cell types, and the assessment of their contribution to the local response to infection. This project will contribute to a better understanding of the early pathogenesis of BRBV in the skin and will inform future vaccine and therapeutic design and development aimed at counteracting this emerging tick-borne virus.

General Microbiology
Ecology/Evolution

Session Leads

Josh Banta, PhD (UTT)

Wei-Chin Ho, PhD (UTT)

Experimental Evolution of *Escherichia coli* in Acidic Environments

Kingsley Amoateng, Wei-Chin Ho

University of Texas at Tyler, Tyler, United States

Abstract

Acidic stress represents a significant pressure that microbes can experience in various natural and anthropogenic contexts, from living within animal guts to food being acidified in factories. However, the rate, trade-offs, and genetic mechanisms of microbial adaptation under persistent acidic stress remains unclear. Here, we experimentally evolved Escherichia coli as a model system to investigate the adaptation to acidic stress. We propagated 12 replicate populations of E. coli for ~1,900 generations in Luria-Bertani media with two pH values (acidic: 4.5–5.0; neutral: ~7.0) with daily transfers, shaking at 37°C. Despite diminishing returns, the evolved populations in the acidic environment exhibited significant increases in the carrying capacity (136 ± 7.3 x 106 CFU/mL), larger than the increase in the neutral environment (60 ± 9.8 x 106 CFU/mL). The competitive fitness of evolved populations in acidic environments increases significantly by 55.8 ± 7.3 %, with no difference in neutral condition (-0.9 ± 1.5 %). The populations in both environments evolved the ability to increase the pH values of medium by ~1.5 after a day of culture. Additionally, the evolved populations in the acidic environment showed more derived biofilm formation and antibiotic resistance than the evolved populations in the neutral condition, suggesting the pathogenicity can evolve as a byproduct of new adaptation to stressful environments. This work enhances our understanding of microbial adaptation and has implications for managing bacteria in acidic conditions.

Bicarbonate Impairs the Evolution of Azithromycin Resistance in *Pseudomonas aeruginosa*

Tuhina Maity, Nicholas Dillon

University of Texas at Dallas, Richardson, United States

Abstract

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CF transmembrane regulator that impair chloride and bicarbonate transport across airway epithelia. This creates a dehydrated, acidic, and viscous airway surface environment that supports chronic infection by *Pseudomonas aeruginosa*, a leading cause of morbidity and mortality in CF patients. Azithromycin (AZM), a macrolide antibiotic with antimicrobial and anti-inflammatory properties, is widely used in CF care. While most resistance studies rely on bacteriological media, our data show that bicarbonate, a physiologically relevant ion, profoundly alters AZM resistance. *P. aeruginosa* is highly resistant in CAMHB (MIC 256 µg/mL), the bicarbonate-free medium required by CLSI guidelines, but very susceptible in bicarbonate-containing RPMI (MIC 1–2 µg/mL). Adaptive laboratory evolution (ALE) in RPMI demonstrated that resistance failed to emerge. Similarly, ALE in CAMHB or RPMI supplemented with bicarbonate showed no resistance development, with MICs remaining at ~2–4 µg/mL, just below the CLSI breakpoint. Substituting bicarbonate with PBS did not reproduce this phenotype, indicating that bicarbonate enhances AZM activity through specific physiological effects rather than pH buffering. Whole-genome sequencing of strains evolved in RPMI with bicarbonate consistently revealed mutations in *mexY*, a component of the mexXY-oprM efflux system. Structural analysis suggested these mutations cause loss of function in MexY, though the relationship between efflux disruption and AZM resistance remains unclear. Notably, bicarbonate-mediated suppression of resistance was reproducible across replicates and extended to *Staphylococcus aureus* and *Acinetobacter baumannii*, indicating a conserved physiological constraint on resistance evolution across species.

From Anemone to Coral: Establishing *Nematostella vectensis* as a Translational Model for Coral Disease Research

James Campbell, Tanya Brown

University of Texas at Tyler, Tyler, Texas, United States

Abstract

Coral reefs, ecosystems vital to marine biodiversity and ecosystem services, are facing decline due to a combination of anthropogenic and environmental stressors. Threats like bleaching, mechanical destruction, and disease have been key drivers of this decline. With their loss, we will also see a decline in biodiversity, economic benefits, and coastal security. Because of the greater prevalence of destructive diseases in recent years, much research has been done to further understand the immune response of the corals, but little has been elucidated. To accelerate immunological research, several translational models have been proposed, including the estuarine anemone, *Nematostella vectensis*. Much of the current work utilizing *N. vectensis* focuses upon microbiome-host interactions. Despite this, the use of the model in immune and disease studies has been limited due to the lack of known lethal bacterial challenges. This project compares bacterially-derived challenges (Lipopolysaccharides) that have historically been used in *cnidarian* immune studies and those known to elicit disease response in select coral species. Overall, LPS appears to inhibit the growth of larvae over fourteen days. However, LPS derived from *Serratia marcescens* proved to have a higher mortality rate. Utilizing the effects on larval development and mortality to compare the bacterial challenges, *S. marcescens* was noted to have a greater effect than that of the other bacteria used in this study. While further work will need to confirm the responses noted are pathogenic stress responses, the data demonstrates the promise of *S. marcescens* as a potential bacterial challenge for future *cnidarian* disease studies.

Implementing Recombineering Coupled with CRISPR/Cas9 Counterselection to Study *Staphylococcus aureus* Mutation Driving Antibiotic Resistance

Liaqat Ali[1,2], May H. Abdel Aziz[1], Shashikant Srivastava[3]

[1]Fisch College of Pharmacy, University of Texas at Tyler, Tyler, United States. [2]2Department of Life Sciences, Tyler Junior College, Tyler, United States. [3]Department of Cellular and Molecular Biology, The University of Texas Health Science Centre at Tyler, Tyler, United States

Abstract

Staphylococcus (S.) aureus is a prevalent opportunistic pathogen with significant morbidity and mortality impact. Manipulating the genome of *S. aureus* is currently a complex, labor-intensive, and challenging process done through allelic exchange methods. We optimized a recently introduced recombineering method, which utilizes bacteriophage-derived recombinase EF2132 to edit the *S. aureus* genome by allowing precise engineering of point mutations using commercially synthesized oligonucleotides. Recombineering to introduce the mutation is coupled with counterselection using clustered, regularly interspaced, short palindromic repeats (CRISPR)/Cas9-mediated endonuclease cleavage to eliminate the wild-type strain by inducing double-stranded DNA breaks in its genome. We have utilized this method to introduce a single point mutation, T331I, I5N, L114S, D242G, G9V, A314T, M192I, S329L, and a double mutation L114S-D242G, to the *S. aureus vraS* gene, which encodes for a histidine kinase in the VraSR two-component system. This system is responsible for sensing and responding to cell wall stress and the mutations were detected in clinically isolated resistant strains, yet it was not clear if it was the driving factor for the resistance phenotype. Our results show that the mutants increase the minimum inhibitory concentration (MIC) of cell wall-inhibiting antibiotics from 2–16 folds compared to the wild type. Additionally, the mutations enhanced the growth rate of *S. aureus* at sub-lethal doses of all three antibiotics, confirming its causal effect on bacterial survival. In summary, we have successfully adapted a rapid and precise genome editing technology for *S. aureus* to reveal the effect of nonsynonymous mutations on antimicrobial resistance.

Uncovering Environmental Nontuberculous Mycobacteria of Respiratory Significance in Northeast Texas

Jenea Travier[1], Rachel Wilsey[1], Kristin Dean[1], Tim Kennedy[2], Jason McGregor[3], Jenny Calk[2], Entze Chong[4], Michael Odell[5], Jashua Banta[5], Jennifer Honda[1]

[1]University of Texas Health Science Center at Tyler, Tyler, United States. [2]University Academy, Palestine, United States. [3]University Academy, Longview, United States. [4]University Academy, Tyler, United States. [5]University of Texas at Tyler, Tyler, United States

Abstract

Nontuberculous mycobacteria (NTM) are environmental opportunistic pathogens that colonize built and natural environments including soil. For susceptible individuals, infection by any of around ten “respiratory relevant” (RR) NTM species (e.g., Mycobacterium abscessus and the Mycobacterium avium complex species) can lead to a chronic, under-diagnosed, and recalcitrant lung disease. Given that NTM are thought to be acquired from the environment, but the distribution of NTM in the environment is sparsely studied, it is important to investigate the diversity of NTM within known geographic hotspots for NTM infections such as Texas. A citizen scientist network of local students participating in the NASA GLOBE program and volunteers performed opportunistic soil sampling between January 2024–July 2025, collecting 172 soils spanning Tyler, Palestine, and Longview – locations chosen to represent urban, suburban, and rural environments, respectively. Of the 172 samples, 12 (7%) were culture positive for NTM. Stratifying by location, Longview 13% (8/60) > Palestine 6% (4/67) > Tyler 0% (0/45). Using partial *rpoB* gene sequencing, RR NTM were not identified, although *Mycobacterium mucogenicum*, *Mycobacterium chelonae*, *Mycobacterium gallinarum*, *Mycobacterium septicum*, *Mycobacterium arabiense* and other non-pathogenic NTM were recovered. This contrasts with our prior studies from the Hawaiian Islands, where RR environmental NTM isolates were often widespread, but aligns with our other data showing the prevalence and composition of environmental NTM isolates vary widely across the continental U.S. Future work to study NTM within freshwater biofilms and dust are on-going and we are developing models to predict environmental NTM prevalence across the varied regions of Texas.

Medical Microbiology
Disease Pathogenesis and Bioinformatics

Session Leads

Shashi Kant, PhD (UTHSCT)

Lianghao Ding, PhD (UTHSCT)

From Petabytes to Privacy: Enterprise Cloud Security for Biomed

Saurabh Srivasta

Long-term colonization selects for genomic adaptations that impact pathogenic potential

Jennifer N. Walker; The University of Texas Health Science Center at Houston

Introduction: Urinary catheters are the most frequently placed medical devices, with million used annually in the US. Their widespread use is due to their efficacy in the clinical management of hospitalized and critically ill patients and at improving the quality of life for millions of individuals, including those with neurogenic bladder dysfunction and the elderly. However, urinary catheters increase the risk of developing symptomatic catheter-associated urinary tract infection (CAUTI), as well as asymptomatic bacteriuria (ASB) – the presence of bacteria in the urine – with virtually all long-term urinary catheters (LTUCs; placed >30 days) colonized. CAUTIs are common, costly, and associated with increased morbidity and mortality. In contrast to urinary tract infections (UTIs) in healthy individuals, which are primarily caused by Escherichia coli (>70%), both CAUTI and ASB are caused by a wide range of uropathogens, with most only making up between 2-10% of cases. Problematically, these uropathogens are often drug resistant, making empiric treatment difficult, and can serve as a reservoir for antimicrobial resistance when they persist as ASB. As urinary catheter use is expected to increase due to the aging population, greater insights into how these other uropathogens cause CAUTI or persist during ASB is urgently needed, especially since most mechanistic studies still focus on UTIs in healthy urinary tracts with Escherichia coli.

Objectives: Using clinically derived, longitudinally collected isolates from individuals with LTUCs, we explored how Staphylococcus aureus, one of the most frequent causes of ASB, persists within the catheterized bladder. Additionally, we investigated how regular catheter exchanges or exposure to anti-staphylococcal antibiotics – the two primary strategies thought to reduced or eliminate microbes from the catheterized urinary tract – impacted that persistence.

Methods: Whole genome sequencing and bioinformatics analyses were performed to determine phylogeny, including sequence types (STs), antimicrobial resistance patterns, and virulence factor carriage. Additionally, we identified single nucleotide polymorphisms (SNPs) accumulating within one virulence factor, the enzyme urease, and using in vitro assays to assess the impact of those SNPs.

Results: A total of 152 S. aureus isolates were longitudinally collected from 20 participants, with an average of 8 strains per person. The majority of the isolates were ST5 and multidrug resistant, encoding an average of 12 antibiotic resistance genes, including the mecA gene, making them methicillin resistant (MRSA). SNP analyses revealed that strains from between participants had tens of thousands of SNP differences. In contrast, strains from within participants had an average pairwise SNP difference of 7, despite being isolated weeks or months apart, suggesting these are the same strain persisting over time. Notably, half of the participants received antibiotic, and all had at least one catheter change during the study. Impactfully, among the SNPs that were observed within clinical isolates, one of the most consistent were found in the urease regulon. In vitro assays indicate that the SNPs within clinical isolates result in increased urease expression and activity.

Conclusions: This study indicates that i) S. aureus persists within individuals with LTUCs, despite antimicrobial exposure and/or urinary catheter exchanges, ii) most isolates are MRSA, which provides a reservoir of antimicrobial resistance within the urinary tract, and iii) as MRSA persists within the catheterized urinary tract, SNPs are selected for that increase pathogenic potential.

Knee Joint-fluid Multiomics in Suspected Pediatric Septic Arthritis Rapidly Identifies Cases with Autoimmune Component

Prithvi Raj

UT Southwestern Medical Center, Dallas, Texas, USA

Background: An accurate diagnosis of septic versus reactive or autoimmune arthritis remains clinically challenging. A multi-omics strategy comprising metagenomic sequencing and immune profiling was undertaken for children diagnosed with presumed septic arthritis to advance clinical diagnoses and care for affected individuals.

Methods: Twelve children with suspected septic arthritis were prospectively enrolled to compare standard of care tests with a rapid multi-omics approach over the period of 9 months. The multi-omics data combined bacterial metagenomics, immune response profiling and highthroughput single cell RNA sequencing on knee joint fluid specimens. The diagnostic value of the multi-omics was ascertained relative to standard of care culture and PCR-negative results.

Results: Ten children with suspected primary septic arthritis and two with acute hematogenous osteomyelitis (AHO) diagnoses were assessed. Joint fluid bacterial cultures were positive for 6/12 (50%) patients, consistent with elevated inflammatory markers (IL-4, IL-6, IL-17A, TNF-a, etc.). Metagenomic bacterial sequencing results were 100% concordant with the culture results. Six patients were culture- and PCR-negative. Multiomics analyses of the 6 culture negative patients established that 2/6 culture-negative children had inflammatory arthritis with potential Juvenile idiopathic arthritis (JIA) and 1 had post-Streptococcal Reactive Arthritis. The children without any bacteremia had autoantibodies (IgGs) in the joint-fluid targeting several nuclear antigens (i.e., *dsDNA, histones, Jo-1, scl-70, Ro/SS-A, SmDs, CENP-A* along with non-nuclear antigens i.e. *Albumin, Collagens, Myosin, Laminin, etc.* Single cell transcriptomics confirmed an abundance of $CD4^+$ follicular helper T (Tfh), CD8+ T cells and B cells in the autoantibody positive subjects. The combination of 16S DNA sequencing ($p=0.006$), cytokine assays ($p=0.009$) and autoantibody profiling ($p=0.02$) were significantly distinct between those children with and without infections. This improved the diagnostic confidence for 9 of 12 (75%) children, key for treatment decisions. .

Conclusions: The multiomics approach rapidly identified children with bacterial or autoimmune inflammatory conditions, improving diagnostic and treatment strategies for those with presumptive septic arthritis.

Ebola Virus Transforms Migratory Properties of Macrophages to Promote Virus Replication and Spread Through Tunneling Nanotubes

Olena Shtanko[1], Carson Leavitt[2], Marija Djurkovic[2]

[1]Texas Biomedical Research Institute, San Antonio, United States. [2]University of Texas Health Science Center, San Antonio, United States

Abstract

Ebola virus (EBOV) causes increasingly more frequent outbreaks of severe disease, for which approved countermeasures are limited to targeting extracellular viral particles. We recently reported that, in addition to the established model of spread by the cell-free form, EBOV propagates replication by intercellular movement of viral nucleocapsids through tunneling nanotubes (TNTs), thus escaping current therapeutics. The virus-induced increase in the TNT number is prominent in macrophages, a primary cell target of the virus, indicating that the escape occurs early during infection. The processes governing EBOV-TNTs interactions are unknown. Here, we show that EBOV exploits podosomes, mechanosensitive adhesive structures used by macrophages to migrate through tissues, to promote virus spread through TNTs. EBOV altered macrophage locomotion and degradation of 3D matrices, suggesting that the virus transforms podosome functions. Using high-resolution 3D microscopy, we identified the EBOV polymerase complex components as factors critical for the transformation, suggesting the involvement of podosomes in EBOV RNA synthesis. Indeed, depleting macrophages of podosome regulators blocked EBOV replication, and the podosome components coimmunoprecipitated with viral replication complexes, supporting the premise. TNTs isolated by laser microdissection and analyzed by mass spectrometry revealed a powerful presence of the podosome machinery in the infected TNTs, indicating its recruitment by the virus during intercellular transfer. We propose that the EBOV replication complex engages the macrophage podosome machinery to promote infection spread by two routes: to facilitate gene expression and amplification of viral genome and to transport replication units through TNTs.

Genomic and Phenotypic Surveillance of *Pseudomonas aeruginosa* Cystic Fibrosis Isolates for Cefiderocol Nonsusceptibility and Hypermutability

Donghoon Alex Kang[1], Philip Thornton[1], George Gulde[1], Rodrigo Baptista[1], Natalia Kirienko[2], Carolyn Cannon[3], William Miller[1]

[1]Houston Methodist Research Institute, Houston, United States. [2]Rice University, Houston, United States. [3]Texas A&M University Health Science Center, College Station, United States

Background: *Pseudomonas aeruginosa* is a Gram-negative pathogen that is the leading cause of chronic lung infections in cystic fibrosis (CF) patients. For difficult-to-treat, resistant (DTR) isolates, one of the last-line antibiotics is cefiderocol (FDC). However, we recently reported that clinical strains frequently exhibit FDC nonsusceptibility (FDC-NS) even without prior exposure to the antibiotic. We aimed to explore the prevalence and genomic determinants of FDC-NS in *P. aeruginosa* strains from CF patients.

Method: We screened 64 CF isolates for FDC-NS and hypermutability by selecting strains on 8 µg/mL FDC or 300 µg/mL rifampicin. Mutations in previously-characterized FDC resistance genes were identified from whole genome sequences.

Results: 19% (n=12/64) of strains were identified as FDC-NS. There was significant enrichment of hypermutators (primarily *mutS/L* mutants) among FDC-NS isolates. FDC-NS was not significantly associated with resistance to other antipseudomonal β-lactam agents, but there was significant enrichment of *oprD* mutations (which contribute to carbapenem resistance) and mutations in the *pir* catechol siderophore uptake pathway among FDC-NS strains. We performed whole-genome, short-read, deep-sequencing in analogous, hypermutable respiratory isolates and observed widespread mutations in TonB-dependent siderophore receptors and their regulatory elements (*pirR*, *pirA*, *piuA*, *piuC*, *pchR*, *fptA*) following FDC exposure, supporting our model of FDC-NS where hypermutability drives the emergence of drug-resistant subpopulations. FDC treatment with the β-lactam enhancer drug zidebactam suppressed the emergence of these TonB receptor mutants.

Conclusions: Hypermutability is a risk factor for FDC-NS. Additional therapeutic strategies such as the combination of FDC and zidebactam could help combat this resistance mechanism.

Sirtuin Modulation Alters HIV Replication in Primary Human Immune Cells and Humanized Mice

Rajesh Mani[1], Alejandro Bohorquez[1], Adduri SitaRamaRaju[1], Mounika Duggineni[1], Andy Omeje[1], Guohua Yi[1], Chinnaswamy Jagannath[2]

[1]The University of Texas Health Science Center at Tyler, Tyler, United States. [2]Houston Methodist Research Institute, Houston, United States

Abstract

Host-mediated factors play important roles in HIV replication and immune response, and they are promising targets for developing adjunctive therapeutic strategies. Earlier studies have reported the link of sirtuin protein's role in controlling the intracellular pathogens, however their role in HIV infection is still largely unknown. We performed in-vitro experiments using human primary macrophages and CD4+ T cells infecting with HIV, treating with either Sirtinol or Sirreal2 (Sirtuin2 inhibitor) and Resveratrol (Sirtuin1 activator) either alone, in combination of both or combined with antiretroviral cART drugs. We measured the viral load, viability and activation markers and cytokines expression. We found that inhibiting Sirtuin2 using Sirtinol and SirReal2 significantly reduced the viral load after 7days of infection. Similarly, activation of Sirtuin1 activity with resveratrol notably reduced the viral. Remarkably, the combination of Sirtuin2 inhibitor and Sirtuin1 activator further reduced the viral load in both the macrophage and CD4+ T cells cultures after 7 days of infection. We also performed in-vivo study in NSG-SMG3 humanized mice co-infected with HIV and Mtb, treated with Sirtinol alone or with cART. We found that Sirtinol alone reduced Mtb growth, while combining sirtinol with cART synergistically reduced the viral load significantly after a month of infection. Together our in-vitro and in-vivo studies show that sirtuin modulation plays a critical role in HIV infection outcomes and host mediated immune response. Furthermore, these results warrant further exploration of Sirtuin based host therapies for HIV infection including studying the synergistic effect with antiretrovirals and exploring the underlying molecular mechanisms.

General Microbiology
Animal Models and Pharmacology

Session Leads

Galina Florova, PhD (UTHSCT)

May Abdelaziz, PhD (UTT)

Streptococcus pneumoniae–Induced Rabbit Model of Empyema Reveals the Contribution of TREM-1 in Pleural Inflammation

Jincy Jacob[1], Christian Jordan[1], Krishna Sarva[1], Sunil Christudas[1], Ali Azghani[2], Galina Florova[1], Andrey Komissarov[1]

[1]UT School of Medicine, Tyler, United States. [2]University of Texas at Tyler, Tyler, United States

Abstract

Empyema, a severe complication of pneumonia, trauma, and surgery, is characterized by excessive inflammation, progressive tissue damage and purulent fluid buildup, ultimately advancing to fibrotic remodeling and pleural thickening. Despite advances in treatment, the global incidence of empyema continues to rise, highlighting the need for a deeper understanding of the immune mechanisms that drive its progression. Triggering Receptor Expressed on Myeloid Cells-1 (TREM-1) is an innate immune receptor that amplifies inflammatory responses through synergistic activation with Toll-like receptor pathways. We hypothesized that TREM-1 plays a role in pleural pathogenesis. To test the hypothesis, we used a *Streptococcus pneumoniae*–induced rabbit model of empyema that closely mimics the clinical condition in humans. Rabbits developed progressive pleural infection with distinct stages, including exudative, fibrinopurulent, and organized phases, accompanied by marked leukocyte infiltration, fibrin deposition, and increased pleural thickening. TREM-1 expression was significantly upregulated in both pleural tissue and infiltrating immune cells, with soluble TREM-1 detectable at elevated levels in pleural fluid. Acute empyema rabbit model showed higher TREM-1 expression and inflammatory cytokines (IL-6, TNF- α), and chemokine (IL-8) release, while the advanced model showed elevated TGF-β. In summary, our data confirm that TREM-1 is involved in the inflammatory cascade in empyema and contributes to pleural tissue damage and fibrosis. Our rabbit model of empyema recapitulates human empyema and can serve as a relevant preclinical model for evaluating TREM-1–targeted anti-inflammatory therapies.

Protocol for Click Labeling of HIV-1 Envelope on Amber-Free Virions Prepared Using Genetic Code Expansion

Wang Xu, Narendra Kumar Gonepudi, Maolin Lu

University of Texas at Tyler Health Science Center, Tyler, United States

Abstract

This protocol describes the steps to produce replication-incompetent HIV-1 virions, which are surface-decorated with clickable envelope proteins using HEK293T cells, followed by fluorescent labeling via click chemistry. Genetic code expansion via amber suppression and click chemistry enables site-specific incorporation of ncAAs into proteins of interest. We generate an amber-free HIV-1 provirus to enable precise genetic code expansion and site-specific incorporation of ncAAs at amber stop codon sites in Env. In our illustrations, clickable Env on amber-free virions incorporates two ncAAs (TCO*A) for dual-color labeling. These steps also apply to preparing viral particles with the appropriate plasmids for single-color labeling. Other ncAAs besides TCO*A function similarly with proper optimization. Dual-color labeled viral particles generated using this protocol suit single-molecule FÖrster resonance energy transfer (smFRET) imaging, while single-color labeled particles support related fluorescence imaging studies using confocal, TIRF, or super-resolution microscopies. This protocol can be adapted to other HIV-1 proteins or to other virus particles (e.g., SARS-CoV-2) and their surface glycoproteins, with necessary adjustments for each system. For example, sites for amber codon insertion into the target proteins, ncAA incorporation efficiency, and virus purification steps should be considered for the viral protein of interest. This protocol describes small-scale virus production and fluorescent labeling. Scaling up is possible by adjusting reagent ratios (plasmids and transfection reagents) according to the culture volume and number of cells in each step and optimizing transfection conditions and purification methods. This work has been published in STAR Protocols.

Mechanisms Underlying PAI-1 Targeted Fibrinolytic Therapy in a Rabbit Model of *Streptococcus pneumoniae*–Induced Empyema

Sunil Christudas[1], Jincy Jacob[1], Krishna Sarva[1], Christian J. DeVera[1], Galina Florova[1], Paul Declerck[2], Andrey Komissarov[1]

[1]University of Texas at Tyler School of Medicine, Tyler, United States. [2]Laboratory for Therapeutic and Diagnostic Antibodies, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium

Abstract

Empyema is a serious condition characterized by the accumulation of pus in the pleural cavity, and its incidence is rising in the US and globally. Plasminogen activator inhibitor-1 (PAI-1), a key inhibitor of tissue-type (tPA) and urokinase-type (uPA) plasminogen activators, is markedly overexpressed in empyema pleural fluid and limits the efficacy of intrapleural fibrinolytic therapy (IPFT). Using a rabbit model of *Streptococcus pneumoniae*-induced pleural injury, we demonstrated that combination of PAI-1–neutralizing monoclonal antibodies (mAbs) with tPA or uPA enhanced the efficacy of IPFT. The *S. pneumoniae*–induced rabbit model of empyema closely recapitulates the clinical condition observed in humans. To investigate the underlying mechanisms, rabbit primary mesothelial cells (RPMC) and a human mesothelial cell line (Met5A), activated with TGF-β, were treated with PAI-1–neutralizing mAbs (MA-33H1F7 and MA-8H9D4). Analysis of conditioned media and cell lysates showed significant reductions in PAI-1 and α-SMA protein expression. Immunofluorescence confirmed decreased PAI-1 and α-SMA in RPMC and reduced PAI-1 in Met5A. Gene expression analysis also revealed downregulation of SERPINE1 (PAI-1) and ACTA2 (α-SMA) in RPMC, and SERPINE1 in Met5A. These findings highlight novel mechanisms by which PAI-1-neutralizing mAbs may affect PAI-1 and α-SMA expression in mesothelial cells.

Investigating the Role of Defensins in Urogenital Epithelial Defense Against Pathogens

Maliha Batool[1], Nicole De Nisco[1,2], Xintong Dong[1]

[1]The University of Texas at Dallas, Richardson, United States. [2]The University of Texas Southwestern Medical Center, Dallas, United States

Abstract

Urinary tract infections (UTIs), primarily caused by uropathogenic *Escherichia coli* (UPEC), are among the most common bacterial infections globally. Rising resistance to frontline antibiotics like trimethoprim/sulfamethoxazole has led to higher recurrence rates and chronic urinary dysfunction, emphasizing the need for alternative therapies. This study explores the role of defensins—key antimicrobial peptides—in both urinary and vaginal tract immunity. The overarching goal of the study is to use a conditional knockout (Def cKO) mouse developed in our lab to delete the defensin gene cluster specifically in bladder and vaginal epithelium and evaluate the necessity of defensins in anti-UPEC immunity. We evaluate how defensin deficiency impacts the animal's ability to clear UPEC through bladder CFU enumeration at various time points in Def cKO versus WT mice in a well-established model of acute bladder infection. Using qPCR and RNA-fluorescence in situ hybridization (FISH) we also evaluate defensin expression dynamics and identify the epithelial subtypes in the bladder, urethra and vagina that express distinct defensin subsets in both naïve and infected mice. Finally, we analyze the immune modulatory functions of defensins in the bladder via flow cytometry and single cell RNA sequencing analysis of immune cell populations in WT and *Def* cKO UPEC-infected mice. These experiments will reveal the impact of defensins on the overall bladder immune landscape. In summary, this study will reveal how defensins contribute to bladder and vaginal epithelial defense, cellular immunity, and immune modulation during UTI. These findings may inform defensin-based therapies for urogenital infections.

Modeling Pleural Fibrosis in Mice: Mechanistic Insights and Translational Potential

Wenyi Qin, Ezinne Osuji, Shuzi Owens, Soyemi Olamipejo, Vijaya Mohan Rao Lella Torry Tucker, Shiva Keshava

University of Texas Health Science Center at Tyler, Tyler, United States

Abstract

Pleural fibrosis (PF) poses a major clinical challenge due to progressive scarring of the pleura, loss of lung compliance, and lack of effective therapies. Current interventions are limited, often invasive, and largely ineffective, reflecting gaps in our understanding of the molecular pathways driving PF. Developing robust preclinical models is therefore essential to dissect pathogenic mechanisms and accelerate discovery of novel therapies. We have established a suite of complementary murine models that mirror diverse etiologies of pleural injury leading to fibrosis. First, a *Streptococcus pneumoniae*–induced empyema model replicates infectious pleural injury and post-infectious fibrotic remodeling, the most common cause of PF in humans. Second, a carbon black–bleomycin model simulates chemically induced pleuritis, providing a fibrogenic stimulus that results in localized scarring. Third, pleural administration of a TGF-β–expressing adenovirus delivers a sustained profibrotic signal, producing robust, reproducible fibrosis. These models have enabled us to test candidate pharmacological inhibitors and evaluate strategies to attenuate pleural remodeling. In recent studies, we introduced a novel approach by administering extracellular vesicles (EVs) isolated from human exudative pleural effusions into mice. This system allows us to investigate whether EV cargo can directly drive PF and to identify potential molecular mediators of fibrosis *in vivo*. Together, these models capture the major clinical contexts of PF-including infection, chemical injury, cytokine-driven signaling, and EV-mediated communication-thereby providing a powerful translational platform. The emerging EV-based strategy, in particular, offers unique opportunities to probe intercellular signaling in pleural fibrogenesis and to reveal novel therapeutic targets for this devastating condition.

Genome Analysis and Dormancy of *Tersicoccus phoenicis*, a Spacecraft Cleanroom Isolate

Madhan R Tirumalai, Sahar Ali, George E Fox, William R Widger

University of Houston, Houston, United States

Abstract

Spacecraft are often built under strict planetary protection guidelines to prevent forward contamination. Yet, despite rigorous cleaning, spacecraft assembly cleanrooms (SACs) harbor resilient microbes. While most are spore-formers, a smaller subset of non-spore-forming actinobacteria, especially within *Micrococcales*, is repeatedly isolated. Their survival strategies remain poorly understood. We investigated *Tersicoccus phoenicis* DSM 30849 (strain 1P05MA), a non-spore-forming SAC isolate. It has a generation time of ~3–3.5 hours. Its draft genome shows a 3.21 Mb assembly (132 scaffolds) with 3,185 predicted genes, including 3,128 protein-coding sequences, 46 tRNAs, and 104 pseudogenes. Comparative analysis with three other *Tersicoccus* genomes revealed 492 unique genes, including toxin–antitoxin systems, phage elements, transporters, DNA-binding proteins, and many hypothetical proteins. Notably, the genome encodes two full-length resuscitation-promoting factor (Rpf) homologs, likely functional in reviving dormant cells. This led to our hypothesis that this strain can both enter and exit dormancy. We evaluated this hypothesis experimentally. We show for the first time that *T. phoenicis* enters dormancy under nutrient starvation and can be resuscitated by Rpf, paralleling mechanisms described in *Micrococcus luteus*. Additionally preliminary UV254 irradiation studies revealed sharp declines in growth after 5 minutes of exposure. However residual survival was observed even after 60 minutes. These findings expand our understanding of non-spore-forming SAC actinobacteria by linking genomic features to dormancy-associated survival. Characterizing such strategies provides new insights into microbial persistence in SACs and has direct implications for planetary protection and long-duration space missions.

Microbiology Research Models

Session Leads

Tanya Brown, PhD (UTT)

Jon Seal, PhD (UTT)

Documentation of Environmentally Acquired *M. intracellulare* subsp. *chimaera* Pulmonary Disease Soon After Bronchiectasis Onset: a case study

Chelsea Raulerson[1], Rachel Wilsey[1], Liang-Hao Ding[1], Christian Castaneda[2], Jamie Joyner[3], Jennifer Honda[1]

[1]University of Texas Health Science Center at Tyler, Tyler, United States. [2]University of Hawai'i, Honolulu, United States. [3]Hawai'i Infectious Disease Associates, Honolulu, United Statess

Abstract

Households are recognized sources for nontuberculous mycobacteria (NTM) exposures. However, documenting NTM infection events and establishing links between environmentally acquired NTM pulmonary disease *a priori* for people with bronchiectasis is challenging. A 79 y.o. female living her entire life in Hawai'i volunteered her home a decade earlier for routine environmental sampling to investigate changes in NTM diversity over an extended period. Between 2012-2024, a single volunteer collected 155 environmental samples from the patient's home across 20 sampling events. In 2024, the homeowner developed bronchiectasis, cough productive of thick sputum, and three sputum specimens grew *M. intracellulare* subsp. *chimaera* (MCHIM). Since the patient met the diagnostic criteria for pulmonary disease, patient and their environmental MCHIM isolates were Illumina whole genome sequenced (n=24), single nucleotide polymorphisms (SNPs) were called, and pairwise distances were calculated. Isolates were considered matched if the SNP distances were <20. We found the three MCHIM respiratory isolates matched each other (max distance=11). Of 11 representative MCHIM isolates recovered from the residence, eight matched with respiratory isolates including one collected 11 years prior to the onset of NTM lung disease. These results suggest that the genomic characteristics of NTM are stable over time and that patient susceptibility may be more predictive of disease development. Future work will focus on expanding our dataset to include more patient-resident pairs. While a single case, study results open the door for renewed discussions to answer the long-standing question of which came first, NTM disease or bronchiectasis or *vice versa* by supporting the latter.

Understanding the Role of Infection in Hemothorax Pathology and Treatment: Presentation of a Noninfectious vs Infectious Rabbit Model of Retained Hemothorax

Christian Jordan De Vera[1], Galina Florova[1], Ali O Azghani[2], Krishna Sarva[1], Sunil Christudas[1], Jincy Jacob[1], Oluwaseyi Akiode[1], Jon M Florence[1], Andrey A Komissarov[1], Steven Idell[1]

[1]The University of Texas Health Science Center at Tyler, Tyler, United States. [2]The University of Texas at Tyler, Tyler, United States

Abstract

Hemothorax involves the accumulation of blood in the pleural space due to various etiologies, including blunt or penetrating trauma, iatrogenic causes, bleeding disorders, or the use of anticoagulants. Failure to drain the blood in the pleural space may indicate the development of a retained hemothorax (RH), which carries the risk of infectious pathologies, such as pneumonia or empyema. The presence of infection within the pleural space greatly exacerbates lung injury and accelerates the development of fibrosis; moreover, the procoagulant environment suppresses the endogenous fibrinolytic activity, thus rendering fibrinolytic therapy ineffective. Therefore, understanding the crosstalk between infection and RH is essential for guiding timely intervention and improving patient outcomes. Here, we present a comparative analysis of noninfectious versus infectious rabbit models of RH. RH was induced by delivering a total of 120 mL of rabbit blood in the pleural space via chest tube. After seven days of disease progression, each subject received fibrinolytic therapy to resolve the RH. Bacterial infection was determined by streaking the pleural fluid on a blood agar plate. Outcomes and the presence of infection were determined after 24 hours. Preclinical subjects with bacterial infection demonstrated increased fibrin deposition, pleural thickening, proinflammatory markers, suggesting that pleural injury was more severe in comparison to noninfectious animals. Our findings demonstrate that bacterial infection may lead to worse outcomes for our animal subjects with RH, serving as a promising starting point for understanding the role of infection in RH pathology and management.

Loss of Cell Envelope-Associated Rough Core Genes of Respiratory *Mycobacterium abscessus* Isolates from People with Cystic Fibrosis Compared to Environmental Isolates

Jenna Rogers[1], Chelsea Raulerson[1], Kaelyn Pacpaco[2], Rachel Wilsey[1], Adrian Hornby[1], Liang-Hao Ding[1], Ethan Hill[2], Jerry Nick[3], Yvonne Chan[2], Jennifer Honda[1]

[1]University of Texas Health Science Center at Tyler, Tyler, United States. [2]'Iolani School, Honolulu, United States. [3]National Jewish Health, Denver, United States

Abstract

Mycobacterium abscessus (MABS) is an environmentally acquired opportunistic pathogen that exists as virulent rough or less-virulent smooth morphotypes. However, the reason for morphological change remains ill-defined. Previous RNAseq analyses show variation in gene expression between sets of rough and smooth environmental isolates. The hypothesis that rough and smooth MABS also show important genetic differences that facilitate survival in the lung was tested. Whole genome sequencing data from Illumina short reads and Oxford Nanopore long reads were combined to create high quality assemblies for seven respiratory (four rough; three smooth) MABS isolates from people with cystic fibrosis and 11 (six rough; five smooth) environmental MABS isolates. Of the isolates that exist as smooth-rough pairs, sequences cluster by pairs rather than morphotype, suggesting that the genetic variation between morphotypes is minimal. When examining the differences between genes present in different sets of isolates, three genes in particular, *cwsA*, *ppiA*, and *glpG* were included in the core genes for all subsets except the respiratory, rough isolates. *CwsA* is required for cell wall synthesis and maintenance of cell shape, and *ppiA* contributes to oxidative stress resistance in macrophages for *Mycobacterium tuberculosis*. Of interest, *glpG* encodes a rhomboid protease; when members of this gene family are lost in *Mycobacterium smegmatis,* it results in altered biofilm production and morphology. These findings suggest that the gene loss in respiratory rough isolates may play a role in morphological change. Validation of these results is underway in a larger set of respiratory and environmental isolates to confirm this hypothesis.

Validation of Plasminogen Activator Inhibitor 1 (PAI-1) as a Molecular Target in Three Rabbit Models of Pleural Injury

Andrey Komissarov[1], Ali Azghani[2], Christian De Vera[1], Sunil Christudas[1], Jincy Jacob[1], Krishna Sarva[1], Rebekah Emerine[1], Oluwaseyi Akiode[1], Sophia Karandashova[3], Steven Idell[1], Alexander Sigalov[4], Galina Florova[1]

[1]The Department of Cellular and Molecular Biology, UT School of Medicine, Tyler, United States. [2]The Department of Biology, University of Texas at Tyler, Tyler, United States. [3]Division of Respiratory Medicine, Department of Pediatrics, UCSD, San Diego, United States. [4]SignaBlok, Inc, Shrewsbury, United States

Abstract

The incidence and mortality of empyema have steadily increased over the past decades. Up to 30% of adult patients are poor candidates for surgical treatment or conventional high-dose enzyme therapy. Notably, in humans with empyema, elevated PAI-1 correlated with the severity of both septation and pleural fibrosis, and with an increase in failure of enzyme-therapy. To address the unmet need for a low-dose approach to treat empyema in a cohort of high-risk, elderly patients, we hypothesized that PAI-1 is a molecular target in pleural injury. The hypothesis was tested in three different rabbit models where pleural fibrosis was induced (i) chemically, (ii) by *Pasteurella multocida* or (iii) *Streptococcus pneumoniae*. Unlike in mice, the fibrinolytic system of rabbits is similar to that of humans, and models recapitulate well the development of pleural injury. Intrapleural PAI-1 overexpression was detected in all three rabbit models. An adenoviral delivery of intrapleural human PAI-1 or stabilization of the endogenous PAI-1 resulted in a failure of an effective dose of the fibrinolysin. The doses of plasminogen activators effective in the chemically induced pleural injury were ineffective in the infectious model, where intrapleural levels of PAI-1 are almost an order of magnitude higher. Finally, neutralization of PAI-1 employing three different intermolecular mechanisms increased the efficacy of fibrinolysins up to 8-fold in our models. PAI-1 is a validated molecular target for pleural injury, and clinical trials are needed to test the efficacy of PAI-1 neutralization. NIH/NHLBI Catalyze program and collaboration with industry assist in the bench-to-bedside translation.

Poster Presentation Abstracts

Graduate
General Microbiology

Metagenomic Insights into the Microbiome of *Amblyomma americanum* Ticks Infected with *Rickettsia amblyommatis:* Functional Predictions with PICRUSt2 and Network Analyses

Sabrina N White[1], Dasiel Obregon Alvarez[2], Michael S Allen[1]

[1]University of North Texas Health, Fort Worth, United States. [2]University of Guelph, Ontario, Canada

Poster G-1, Graduate General Microbiology

Purpose: This study investigates the microbiome of adult male and female lone star ticks (*Amblyomma americanum*) and the effects of *Rickettsia amblyommatis* infection. Little is known about the pathogenicity of *R. amblyommatis* or its role within the tick microbiome, but its prevalence in *A. americanum* ticks across Texas and the southeastern United States necessitates further study. By analyzing microbiome differences in ticks collected from a single site over an 11-year period, we will assess how the presence of *R. amblyommatis* influences the broader tick microbiome, predict functional metabolic pathways, and construct microbial interaction networks to identify keystone taxa that shape community structure.

Methods: Ticks were collected from a single site in Palestine, TX, and submitted to the Laboratory over a period of 11 years. DNA was extracted, and samples were screened via PCR for *Rickettsia* spp. Four groups of male and female ticks, previously determined to be positive or negative for *R. amblyommatis*, were randomly selected for analysis (4 groups; N=15 each). The V4 region of bacterial 16S rRNA genes was amplified by PCR in duplicate, pooled, and prepared for sequencing on the Illumina MiSeq platform. Bacterial composition was analyzed using QIIME2. Functional metabolic predictions were generated with PICRUSt2, while microbial interaction networks were inferred through co-occurrence analyses to predict associations among bacterial taxa.

Results: This study will provide insights into how *R. amblyommatis* shapes microbial community structure within *A. americanum* ticks, identify key metabolic functions associated with infection status, and reveal microbial interactions within the microbiome.

Exploring Immune Priming in the Species *Exaiptasia diaphana*

Torrence Campos, Tanya Brown

University of Texas at Tyler, Tyler, United States

Poster G-2, Graduate General Microbiology

Abstract

Vibrio coralliilyticus is a pathogen that infects various coral species across several reef ecosystems and causes mortality within a span of a few days. With increasing temperatures, the pathogen becomes more virulent towards coral. Probiotics such as MCH1-7 have previously been used to treat infected corals before and after exposure to this pathogen. In this study, we are looking at the cnidarians' ability to be primed. Immune priming is the natural way for cnidarians to recognize bacteria from their first encounter and be able to fight off the pathogen. In this study, we will be using a species of sea anemone, *Exaiptasia diapahana,* in place of coral to evaluate the effects of two *V. coralliilyticus* strains: ATCC BAA-450 and OFT6-21. We will also determine if the anemones can be primed using a probiotic. During the preliminary inoculation trials, *V. coralliilyticus* strain OFT6-21 was monitored for ten days after inoculation to monitor stress changes. A concentration of 10^8CFU/ml experienced immediate stress as opposed to the 10^7CFU/ml and 10^6CFU/ml concentrations. Despite stress, throughout the monitoring period the anemones did not have deaths. The BAA-450 strain proved it was more pathogenic at 10^8CFU/ml than OFT6-21 as death occurred by day six and continued to the last day. In total, there were nine deaths. Trials involving probiotic MCH1-7 experienced three deaths at the end of the experiment, but it was the highest concentration level. Based on these results, we will use 10^8CFU/ml for both *V. corallilyticus* strains and 10^7CFU/ml for the probiotic priming dose.

Comparative Analysis of Plasmid Genomes in *Cereibacter* Species

Jaqueline Basurto[1], Madison Schultz[2], Madhusudan Choudhary[1], Anand Karki[1]

[1]Sam Houston State University, Huntsville, United States. [2]Sam Houston State University, Huntsville, United States

Poster G-3, Graduate General Microbiology

Abstract

Ceriebacter (formerly *Rhodobacter*) species is a gram-negative bacterium from the family *Rhodobacteriace,* widely recognized for its metabolic versatility, and ability to thrive in diverse growth conditions, which include aerobic, anaerobic, and photosynthetic. *Cereibacter* plasmids are well known for carrying genes that enable a wide range of metabolic functions and make them valuable for biotechnology applications. The objective of this study is to annotate these plasmid sequences to identify homologous core genes and assess their functional potential. The sequences of 37 plasmids of Cereibacter species were obtained from Plasmid Large Sequence Database (PLSDB) (v.2024_05_31_v2). All 37 plasmids were annotated in Galaxy; similar genes were clustered using Roary (v3.13.0) and Bakta (v1.9.4) for plasmid analysis. Plasmid sequences were screened using Bacterial and Viral Bioinformatics Resource Center (BV-BRC) for their metabolic functions involving antimicrobial resistance, transposition, and pathogenicity. This study provides new insights into the diversity of *Cereibacter* plasmids and their potential roles in their environmental adaptation.

Anthropogenic Impact of Land Development on Soil Microdiversity and Microbial Resistance

Robert Garcia

Texas A&M University in San Antonio, San Antonio, United States

Poster G-4, Graduate General Microbiology

Abstract

This study examines the abiotic, physicochemical, and hydrological impact of land development on microbial diversity and the abundance of antimicrobial-producing organisms. Building on previous research conducted at VIDA, a 600-acre residential development situated near Texas A&M University in San Antonio, which includes site comparisons of Undisturbed, Residential, and Greenway (LID site). Soil samples were collected monthly for a year, with additional samples taken from secondary LID and undisturbed sites to reduce bias and evaluate if patterns observed at VIDA sites were consistent with broader LID and undisturbed sites. Preliminary findings suggest a closer similarity between the Greenway Undisturbed sites, which were similar in abundance of antimicrobial-producing microbes and overall abundance in colony-forming units (CFUs) in VIDA. Soil physicochemical properties will be analyzed to determine their influence on microbial patterns. The study aims to identify which environmental variables have the most significant impact on soil microecology.

LPF1 and LPF2 Are Essential for Enterohemorrhagic Escherichia coli Colonization of the Zebrafish Intestinal Tract

Walter Galdamez, Soumita Dutta, Cecilia Fadhel-Alvarez, Anne Krachler

UTHealth Houston, Houston, United States

Poster G-5, Graduate General Microbiology

Abstract

Enterohemorrhagic *Escherichia coli* (EHEC) is a foodborne pathogen that causes severe gastrointestinal disease and hemolytic uremic syndrome. EHEC employs multiple virulence factors to colonize the host intestine, including the locus of enterocyte effacement (LEE) encoded type III secretion system, intimin, and flagella, yet the role of long polar fimbriae (Lpf) in adhesion and colonization remains less well understood. Long polar fimbriae can mediate bacterial attachment to host cells *in vitro,* yet the regulation and function of its two fimbrial operons, *lpf1* and *lpf2 in vivo* remain poorly understood. We hypothesize that long polar fimbriae subunits LpfA1*,* LpfA2*,* and/or both are essential for effective EHEC adherence and colonization of the zebrafish gut and are regulated in a spatiotemporal manner during infection. Here, we utilize a larval zebrafish (*Danio rerio)* model of foodborne EHEC infection to investigate their contribution to gut colonization. As a vehicle for foodborne infection, we use *Paramecium caudatum,* a unicellular protozoan and a natural prey of larval zebrafish. We demonstrate the major structural subunits of long polar fimbriae, LpfA1 and LpfA2*,* are required for efficient bacterial adherence and persistence within the zebrafish midgut. Deletion of LpfA1*,* LpfA2*,* or both significantly reduced bacterial attachment, leading to lower EHEC recovery within the zebrafish midgut. Fluorescence imaging confirmed reduced gut colonization by *lpf*-deficient strains. These findings provide insight into EHEC pathogenesis and may inform future strategies to mitigate bacterial adhesion and infection.

Prevalence and Species Diversity of Non-tuberculous Mycobacteria in North Texas

Manoj Sah[1,2], Lenin Mahimainathan[1], Muluye Mesfin[1], Andrew Clark[1], Jeffrey SoRelle[1]

[1]University of Texas Southwestern Medical Center, Dallas, United States. [2]University of Texas Health Science Center, San Antonio, United States

Poster G-6, Graduate General Microbiology

Background: Non-tuberculous mycobacterial (NTM) infections are an emerging group of related opportunistic pathogens that resemble tuberculosis (TB) infections and have a wide range of virulence. The variety of causative agents of NTM disease highlights the importance of rapid identification, as it varies by geographic region, and treatment strategies differ. This study aimed to determine the prevalence of NTM recovered in North Texas.

Methods: A retrospective study was conducted between January 1, 2022, to December 31, 2023, including 15,724 pulmonary and extra-pulmonary specimens submitted to Acid Fast Bacilli (AFB) culture. A total of 820 specimens growing with the *Mycobacterial* species were counted for the final analysis. Species prevalence, growth site, and seasonal trends were evaluated at our site.

Results: Among 15,724 AFB cultures, 5.21% (n=820) were positive for 24 species/subspecies of mycobacteria. Of these, 97% (n=795) were NTM and 3% (n=25) were *M. tuberculosis* complex, with an overall NTM prevalence of 5.05%. NTM were isolated from 15.4% (704/4574) of pulmonary and 0.82% (91/11,150) of extra-pulmonary specimens, with 63% (n=514) being slow growing. The most common species were MAC (46%, n=375), *M. abscessus* complex (23.78%, n=195), *M. chelonae* (4.9%, n=40), *M. mucogenicum phocaicum* (3.9%, n=32), and *M. arupense* (2.1%, n=17). MAC showed seasonal peaks in October and lows in July and February, while M. abscessus had no seasonal trend.

Conclusions: Our study found that NTM infections exceeded TB, with MAC and *M. abscessus* dominant. This highlights the need for precise species identification due to geographic and treatment differences.

Phage Peptides at Work: Mechanisms of Membrane Disruption

Michael Debrah, Jolene Ramsey

Texas A&M University, College Station, United States

Poster G-7, Graduate General Microbiology

Abstract

Phages are predators of bacteria that release their progeny by host cell lysis. Lysis requires phages to cross multiple layers of the cell envelope: the OM, the peptidoglycan, and the IM. The holin is responsible for initiating lysis by making holes in the IM. Then, the endolysin degrades the peptidoglycan. Finally, an active OM disruption step is essential for phage release. One phage strategy uses spanin proteins, which induce inner and outer membrane fusion. We recently discovered the disruptins, a class of small cationic phage peptides that disrupt the outer membrane. Unlike spanins, disruptins can both disrupt the OM and cause cell death independent of holin and endolysin activity. We hypothesized that this peptide, called gp28, is effective because it destabilizes the OM by disrupting the interaction between divalent cations and negatively charged phosphate groups in lipopolysaccharide on the cell surface. We are testing this hypothesis from three angles: 1) studying gp28 interaction with LPS, 2) assaying gp28 specificity in bacterial membranes, and 3) leveraging host and phage genetics to identify cellular targets and determinants of resistance. We demonstrate that the activity of gp28 is inhibited by increasing concentrations of LPS in an MIC assay. Although our prior studies suggested that gp28 permeabilized the IM, we show that the permeabilization does not affect the timing of holin activation. Additionally, we show that gp28 has antibacterial activity against a panel of Gram-negative bacteria. These studies on the mechanism gp28 uses to kill Gram-negative bacteria lay the foundation for phage peptide-mediated therapy.

Regulating the End Game: Lysis and Lysis Inhibition in Bacteriophage N4

Michael Awuah[1], Cody Martin[2], Jolene Ramsey[1]

[1]Texas A&M University, College Station, United States. [2]University of Wisconsin, Madison, Madison, United States

Poster G-8, Graduate General Microbiology

Abstract

The lytic life cycle of dsDNA bacteriophages, which infect Gram-negative bacteria like *Escherichia coli*, culminates in an explosive and highly controlled lysis event to release new virions. In bacteriophage N4, this process involves a complex system that can undergo lysis inhibition (LIN), a phenomenon that delays cell rupture for many hours, likely to maximize phage progeny when external conditions are unfavorable. The timing of lysis is primarily controlled by holins, a class of proteins that form pores in the host's cell membrane. N4 phage utilizes two distinct lethal holins: gp63 and gp62. Gp63 acts as a "pinholin," creating small, nanometer-scale holes that lead to rapid and synchronous cell lysis. In contrast, gp62 forms large, micron-scale holes and is associated with a much later, asynchronous lysis characteristic of the LIN state. Experiments using plasmid-encoded lysis proteins showed that gp62 is essential for establishing LIN. When gp63 is inhibited, the phage appears to rely on the slower action of gp62 or a combination of its endolysin and spanin proteins to eventually escape from the LIN state. These findings highlight the critical role of different holins in regulating the timing of lysis, providing the phage with alternative strategies for releasing its progeny based on environmental cues. This research expands our understanding of the sophisticated mechanisms controlling viral life cycles.

Deciphering HSP70 Role in Coordinated Mitochondrial Dynamics and Vesicle-Mediated Egress of Coxsackievirus B

Jayden Aleman

University of Texas at Dallas, Richardson, United States

Poster G-9, Graduate General Microbiology

Abstract

Coxsackievirus B (CVB) is a non-enveloped, positive-sense RNA virus that causes mild respiratory illness but can also result in severe diseases such as myocarditis, pancreatitis, and meningoencephalitis. Recent work has shown that CVB infection induces mitochondrial depolarization and fragmentation, activating mitophagy. During this process, infectious virions can be incorporated into mitophagy-derived vesicles and released, suggesting that CVB exploits mitochondrial quality-control pathways for viral egress. We observed that mitophagy-derived EVs are enriched with 70-kDa heat shock protein (HSP70). Furthermore, CVB capsid protein was detectable within isolated mitochondrial fractions, implicating mitochondria as a potential site of viral replication or assembly. Co-immunoprecipitation confirmed an interaction between HSP70 and VP1, strengthening the evidence for a direct role in viral dynamics. Canonically, HSP70 is recognized as a molecular chaperone that maintains protein folding and supports cellular stress responses, and it has previously been shown to enhance translation of CVB transcripts. However, its association with mitochondria and mitophagy-derived EVs during infection has not been reported. We aim to define how HSP70 contributes to CVB trafficking through mitochondria and its incorporation into vesicles. This work is significant because it identifies a previously unrecognized role for HSP70 at the interface of mitochondrial dynamics and viral egress, providing new insight into non-enveloped virus propagation.

Host Range Analysis of *Staphylococcus aureus*-Infecting Bacteriophages Isolated from Diverse Environments

Alexandra Davis[1], Lilly Westfall[2], Chelsy Mani[2], Tessa Nester[1], Ray Esparza[2], Caden Reynolds[2], Michael Allen[1]

[1]UNT Health, Fort Worth, United States. [2]Memsel, Fort Worth, United States

Poster G-10, Graduate General Microbiology

Abstract

Antibiotic-resistant microbes kill around 5 million people worldwide per year, and deaths due to resistant microbes are anticipated to surpass cancer deaths by 2050. *Staphylococcus aureus* is of particular concern, causing more deaths worldwide (1.1million) than malaria (643,000), breast cancer (700,660), or HIV/AIDS (864,000) in 2019. *S. aureus* is a common commensal skin bacterium and is one of the leading causes of healthcare-associated infections. *S. aureus* is well known for its ability to develop antibiotic resistance via horizontal gene transfer. Compounding the problem, developing new antibiotics is costly, time-consuming, and quickly undermined by new drug resistance, leading to the urgent need for alternative therapeutics. One promising alternative treatment is bacteriophage therapy. Bacteriophages, or simply “phages,” are viruses that specifically infect bacteria in a highly host-specific manner, often infecting only certain strains within a bacterial species. Therefore, to fully employ bacteriophages as an alternative treatment, a group of bacteriophages must be isolated and assembled into a cocktail having infectivity towards a broad range of strains within the species. In this study, phages isolated from diverse environments were tested against a broad range of *S. aureus* clinical isolates acquired from the CDC’s ARBank. Each phage was evaluated for the number of strains it could infect using two different methods, which were then compared for concordance. The results of this study will facilitate the selection of phages with broader host ranges that will improve phage cocktail efficacy.

Computational Prediction of Host–Virus Protein–Protein Interactions

Kexin Pang, Jared Bard

Texas A&M University, College Station, United States

Poster G-11, Graduate General Microbiology

Abstract

Virus infections typically involve extensive protein–protein interactions (PPIs) between viral and host proteins. These interactions enable viruses to exploit host cellular machinery for replication, induce virulence, and disrupt essential host processes such as translation. Identifying host targets of viral proteins is therefore critical for understanding infection mechanisms and can facilitate the development of antiviral therapeutics. Traditionally, PPIs are identified through experimental methods such as yeast two-hybrid assays and mass spectrometry, but these approaches are often time-consuming and labor-intensive and lack molecular detail. In contrast, computational prediction offers a scalable alternative that can provide high-resolution interaction models. In this study, we developed a computational workflow for predicting human–virus PPIs, integrating AlphaFold-Multimer for structural modeling and the SPOC classifier for interaction evaluation. We assessed the performance of our pipeline using a benchmark dataset curated from the STRING Viruses database. Optimization of key parameters is currently underway to improve prediction accuracy. Once validated, we aim to apply this workflow to large-scale screening for viral proteins that may contribute to host translation shut off.

Graduate
Medical Microbiology

Impaired DNA Repair Protein Enhances Biofilm Defenses Against Antibiotics in *Acinetobacter baumanni*

Suman Tiwari, NIcholas Dillon

The University of Texas at Dallas, Richardson, United States

Poster G-12, Graduate Medical Microbiology

Abstract

Minocycline (MIN), a broad-spectrum tetracycline-class antibiotic, is a vital therapeutic option for infections caused by multidrug-resistant *Acinetobacter baumannii* (Ab). Although efflux pumps such as TetB are well-characterized in other bacteria, and commonly linked to tetracycline resistance, they do not fully account for MIN resistance observed in Ab isolates. This suggests the involvement of additional, previously unrecognized mechanisms of resistance. Through machine learning-based computational analysis of over 1,440 Ab clinical genomes, we identified significant correlations between MIN resistance and mutations in *ruvB.* Mutations in *ruvB* have not previously been associated with MIN resistance. We utilized two Ab strains: the multidrug-resistant AB5075 and the antibiotic-susceptible ATCC 19606 to examine how ruvB mutations impact MIN resistance. In both, disruption of *ruvB* led to a substantial increase in MIN resistance and a dramatic enhancement in biofilm formation. Biofilms from *ruvB* disruption were found to be composed of a higher concentration of extracellular DNA (eDNA). During DNA repair by the RuvABC system, RuvA first binds to the DNA at Holliday junctions and then recruits RuvB, which helps drive the movement of the DNA strands. We therefore hypothesize loss of RuvB allows unbound RuvA to stabilize eDNA. Restoring *ruvB* reversed these effects. RuvB mutants also showed increased resistance to other antibiotics, suggesting freeing of RuvA may confer a broad, biofilm-driven, mechanism of resistance in Ab. This previously unrecognized mechanism underscores the role of biofilm physiology in antibiotic resistance and presents new opportunities to develop therapies that extend the effectiveness of existing antibiotics through targeting biofilm regulation.

Salmonella Co-culture Affects Stress Survival of *Campylobacter jejuni* in vitro

Samuel Cannon, Alondra Lugo, Layla Behrens, Anand Karki, Todd Primm

Sam Houston State University, Huntsville, United States

Poster G-13, Graduate Medical Microbiology

Abstract

Prevalence of *Campylobacter* is a continuing challenge for healthcare, eliminating nearly 13,000 quality of life years in the U.S. alone. This prevalence is paradoxical to the fragility of C*ampylobacter* as demonstrated by the niche conditions required for growth. These conditions include a microaerobic environment, high temperatures (37-42° C) and supplementation with blood. The narrowness of *C. jejuni*'s permissible conditions can be rooted in the rudimentary nature of *Campylobacter*'s gene ontology. A stark lack of sugar metabolism, stress resistance, and gene regulation certainly contributes to the weak virulence factors in vitro. This gap between epidemiological prevalence of *Campylobacter* and its frailty has eluded researchers, however; there has turned up a possible lead. Polymicrobial interactions (PMI's) have been of increasing interest. In the past several years more evidence has shown that *C. jejuni*'s virulence factors are enhanceable when cocultured with select microbes i.e. *S. aureus*,*Acanthamoeba polyphaga*, and *Pseudomonas* spp. My thesis proposes that a common contaminant, *Salmonella enterica* subsp. *enterica,* enhances the survival in stress conditions and propensity for growth of *C. jejuni*. Furthermore, with the addition of attenuated and cell-free cultures, the mechanisms attributable can be extracted in the process of characterizing PMI mediated enhancement of virulence.

Vascular Endothelial Growth Factor Impacts Blood Brain Barrier Integrity During Group B *Streptococcal* Infection

Natalie G. Alexander[1,2], Benjamin T. Klemp[1], Kylie A. Buchannan[2], Alexis Rickenbacher[2], Alexandra E. Meyer[2], Brandon J. Kim[1,2]

[1]The University of Texas at Dallas, Richardson, United States. [2]The University of Alabama, Tuscaloosa, United States

Poster G-14, Graduate Medical Microbiology

Abstract

Neonatal meningitis is a severe infection of the CNS that occurs when the opportunistic pathogen Group B *Streptococcus* (GBS) is able to cross the highly selective blood-brain barrier (BBB). The BBB is comprised of specialized brain endothelial cells (BECs) that possess complex tight junctions and serve as the interface between the circulation and the central nervous system (CNS). An intact BBB is essential for the maintenance and regulation of the neural microenvironment. The mechanisms by which GBS invades the CNS are not completely known. Vascular Endothelial Growth Factor (VEGF) is a secreted angiogenic mitogen known to increase vascular permeability and disrupt the BBB through promotion of leaky blood vessels. RNA sequencing data revealed that *VEGFA* was upregulated during GBS infection in BECs. Using two *in vitro*BBB models, we confirmed RNA sequencing data showing *VEGFA* is upregulated during infection. To demonstrate that VEGF is contributing to BBB disruption, we treated BECs with recombinant VEGF (rVEGF) and observed a decrease in tight junction continuity and expression, demonstrating VEGF itself is able to reduce BBB properties. Additionally, we performed preliminary studies to discover the signaling pathway VEGF is activated through during GBS infection. Our preliminary data demonstrates that the inhibition of MEK/ERK signaling results in a decrease of *VEGFA* expression during GBS infection. Additionally we saw that inhibition of MEK/ERK signaling significantly decreased GBS invasion of BECs. Together these data demonstrate GBS increases *VEGFA* expression at the BBB which may act as a mechanism contributing to barrier dysfunction.

Spatial Mapping of Bladder Innervating Nociceptor Subsets in Mouse Bladder and Their Potential Role in the Neuro-Immune Axis During Urinary Tract Infection

Ridma Sirimanna, Kathleen Halliburton, Xintong Dong

University of Texas at Dallas, Richardson, United States

Poster G-15, Graduate Medical Microbiology

Abstract

Peripheral sensory neurons innervating visceral organs are increasingly recognized as modulators of infection and immunity. The bladder is innervated by sensory neurons that contribute to normal bladder functions such as voiding, but the specific subtypes of sensory neurons in the bladder have not been carefully characterized and their anatomical distributions unclear. Further, the functions of sensory neurons during urinary tract infections (UTI) remain incompletely understood. In this study, we performed comprehensive nociceptor profiling of the bladder using Cre-dependent tdTomato reporter mouse lines. Phosphoinositide interacting regulator of TRP (Pirt)-Cre/tdTomato mice that drives tdTomato expression in all sensory neurons served as a reference, revealing widespread innervation across the bladder. We found that Mas-related G protein-coupled receptor Mrgprd, and calcitonin gene-related peptide (CGRP) expressing nociceptor populations were present prominently in the urothelium layer of the bladder. In contrast, sodium channel Nav1.8 positive nociceptors were expressed primarily in the muscle layer of the bladder and itch specific Mrgpra3 nociceptors were less abundant in the bladder. To assess the impact of nociceptor ablation on the neuro-immune axis during UTI, we ablated Nav1.8+ nociceptors using the expression of Cre-dependent diphtheria toxin A fragment (DtA). Upon infection with UTI89, these mice exhibited a trend of reduced bacterial burden compared to controls, suggesting that Nav1.8+ nociceptors may modulate infection dynamics. Together our findings show the spatial distribution of bladder innervating nociceptor populations and provide preliminary evidence for Nav1.8+ nociceptor population’s contribution to UTI outcomes, establishing a foundation for future studies of the bladder neuro-immune axis.

Campylobacter in Polymicrobial Environments: Role of Nutrient Composition

Stanlee Brandt, Todd Primm, Anand Karki

Sam Houston State University, Huntsville, United States

Poster G-16, Graduate Medical Microbiology

Abstract

Campylobacter jejuni is a leading cause of foodborne gastroenteritis worldwide. While isolated *Campylobacter* has been extensively studied under laboratory conditions in pure culture, it naturally coexists with other bacteria during infections and in environmental settings, which may influence its pathogenesis and survival. *Enterococcus faecalis*, frequently found as a co-contaminant with *Campylobacter* in retail poultry products, may affect *Campylobacter's* virulence and stress tolerance, though this relationship remains poorly understood. *E. faecalis* increases the lethality of *Campylobacter* during oral infection of *Galleria mellonella.*The dynamics of *C. jejuni* and *E. faecalis* co-culture in the presence of diverse nutrient compositions are under investigation and may vary, as suggested by preliminary findings.

Transcriptomic Analysis of Coxsackievirus B3 Infection in Induced Pluripotent Stem Cell-Derived Brain-Like Endothelial Cells

Taryn Keyzer, Jon Sin, Brandon Kim

The University of Texas at Dallas, Richardson, United States

Poster G-17, Graduate Medical Microbiology

Abstract

The Blood Brain Barrier (BBB) is a highly specialized cellular barrier composed of brain endothelial cells (BECs) that prevent the passage of toxins and pathogens into the Central Nervous System (CNS). Coxsackievirus B3 (CVB3) is a non-polio enterovirus and is a leading cause of viral meningitis, however, the mechanism through which CVB3 penetrates the BBB is unclear. Our lab models the BBB using induced-pluripotent stem cell derived brain-like endothelial cells (iBECs), as they recapitulate important barrier properties such as tight junctions and transendothelial electrical resistance (TEER). We have demonstrated, through RNAseq, that CVB3 elicits a significant change in iBEC gene expression at 5 days post infection (PI), including upregulation of antiviral and proinflammatory genes and enrichment of MAPK signaling. We validated the RNAseq results by running qPCR on highly upregulated antiviral and proinflammatory markers, as well as an ELISA for the chemokine RANTES/CCL5. For further study of the enrichment of MAPK signaling, we inhibited the MEK/ERK pathway with U0126. At 5 days PI, we observed an increase in Green Fluorescent Protein (GFP) and Viral Capsid Protein (VP1) abundance in U0126-treated CVB3 infected cells, but a decrease in released virus. We hypothesize that MEK/ERK signaling plays an important role in the response against CVB3 infection in the BBB. Future work aims to further observe inhibition of the MEK/ERK pathway and other branches of MAPK signaling.

Generation of a Stable Knock-In of *Streptococcus agalactiae* Using a CRISPR/Cas12 System

Darla Delozier, Cody Cutts, Brandon Kim

The University of Texas at Dallas, Richardson, United States

Poster G-18, Graduate Medical Microbiology

Abstract

Streptococcus agalactiae (Group B Streptococcus, GBS) is a Gram-positive opportunistic pathogen and is the leading cause of neonatal meningitis worldwide. There is currently no vaccine available, so understanding the mechanisms behind GBS pathogenesis is paramount to developing targeted treatments. GBS contains an endogenous CRISPR/Cas9 gene editing system, and therefore is not susceptible to external editing using the Cas9 protein. To overcome this, we used the Cas12 protein paired with targeted guide RNAs to introduce genomic changes to GBS. To visualize GBS during infection we sought to knock in the gene encoding Green Fluorescent Protein (GFP) into the GBS chromosome, creating a fluorescent strain of GBS. Once generated the GBS-GFP strain will be characterized to ensure there is no confounding phenotype by conducting growth curves in various media, and cell-based adherence/invasion assays compared to the wild-type GBS. This stable knock-in of GFP eliminates the risk of plasmid loss during replication, and makes a fluorescent GBS strain more robust and scalable. In the future, this Cas12-based editing system will be used to introduce various fluorescent markers, such as RFP, enabling co-visualization with host cell markers, as well as expanded to deletion of complementation of functional genes to better understand the roles of bacterial virulence factors.

Identification of a Novel Virulence Factor Contributing to *Streptococcus agalactiae* Interaction with Brain-like Endothelial Cells

Bryan Estrada, William Cutts, Brandon Kim

The University of Texas at Dallas, Richardson, United States

Poster G-19, Graduate Medical Microbiology

Abstract

Streptococcus agalactiae (GBS), a Gram-positive opportunistic pathogen that asymptomatically colonizes the vaginal tract of up to 30% of women. GBS is the leading cause of neonatal meningitis, a life-threatening illness characterized by inflammation of the meninges that occurs when GBS interacts with brain endothelial cells and disrupts the blood-brain barrier. Despite the adoption of intrapartum antibiotic prophylaxis, 3-25% of infant cases may result in death or the development of permanent neurological impairments. Therefore, understanding the mechanisms of infection is necessary for the development of therapeutics. The discovery of GBS factors that contribute to bacterial meningitis has relied on transposon mutant screens, which could have inherent bias. To overcome this, we have performed an RNA-seq analysis of the GBS transcriptome during interaction with induced brain endothelial cells (iBECs), revealing factors that are significantly upregulated compared to the non-interacting GBS, enabling the discovery of novel virulence factors. Potential Virulence Factor (PVF) encodes an uncharacterized protein containing a surface anchor C-terminal LPXTG motif, indicating a possible role in interaction with host cells. Using CRISPR interference, PVF knockdowns were generated and preliminary adherence and invasion assays were conducted on hCMEC/D3s *in vitro*. Compared to control, cells infected with PVF K/Ds had a significant reduction of invasive and adherent bacteria. Additionally, iBECs infected with PVF K/Ds had a reduction in adherent bacteria *in vitro*. Generation of a ΔPVF mutant using a CRISPR-Cas12 system is currently ongoing and will enable the characterization of a potential novel GBS virulence factor.

The Role of Stress Granules and G3BPs in the Translation Regulation During Viral Infection

Thu Nguyen, Jared Bard

Texas A&M University, College Station, United States

Poster G-20, Graduate Medical Microbiology

Abstract

During viral infection, host cells shut down global protein synthesis, followed by the formation of stress granules (SGs) that sequester mRNAs and translation machinery as part of the antiviral defense. G3BP1, a central SG scaffold protein, drives this assembly, and SGs are thought to restrict viral access to host translation. However, many viruses target G3BP1 to block SG formation and, in some cases, even recruit it to promote their own translation. Chikungunya virus (CHIKV), a mosquito-borne alphavirus responsible for over 431,417 cases worldwide in 2024, relies heavily on G3BPs, as viral translation and replication are severely impaired in G3BP-deficient cells. Yet, how G3BP1 supports viral replication remains unclear. A central question is whether SGs and their components actively modulate both host and viral translation. To investigate this, we employ a G3BP1 inhibitor (G3I) that binds the NTF2L domain and disrupts interactions between G3BP1 and other SG-associated proteins, as well as between G3BP1 and viral proteins. Translation and replication will be assessed using qPCR, polysome profiling, imaging, and viral titers. We predict that G3I treatment will impair both host and viral translation, highlighting G3BP1 as a critical regulator of protein synthesis during infection.

Toxin-antitoxin PasTI is Important for Virulence and Cell Persistence in *Escherichia coli*

Vitoria Bueno Vilela Silveira, Kenneth Keiler

University of Texas at Austin, Austin, United States

Poster G-21, Graduate Medical Microbiology

Abstract

Recurrence is the major contributor to the financial and social burden of urinary tract infections (UTIs). It is mainly caused by *Escherichia coli* that persists in the host after antibiotic treatment. One potential solution is to use anti-virulence drugs that limit virulence without inhibiting bacterial growth. From a screen of mutations, the toxin-antitoxin (TA) PasTI genes disrupted virulence and played a role in the formation of persister cells in a clinical isolate, CFT073 *E. coli*. Our data suggest that PasTI affects another virulence factor, *trans*-translation, which is the major ribosome rescue pathway in bacteria. We analyzed the *trans*-translation activity between WT and Δ*pasTI* CFT073 *E. coli* by using an *in vivo* fluorescent reporter (mCherry) that is targeted for *trans*-translation activity. We observed that there is low *trans*-translation activity in the absence of PasTI. In addition, PasTI overlaps with the promoter of the SmpB gene, which is one of the two components of *trans*-translation. Since TA systems generally autoregulate, it is possible that PasTI autoregulation also influences SmpB expression. To test this hypothesis, I will measure the transcription levels of SmpB between WT and the Δ*pasTI* mutant by qPCR. If the levels of SmpB transcript are higher in WT than in the Δ*pasTI* strain, it suggests that PasTI is required for the expression of SmpB.

Investigating the Condensome Network During CHIKV Infection with a FACS-Based Method

Diyun Sun, Maxine Cañelas, Jared Bard

Texas A&M University, College Station, United States

Poster G-22, Graduate Medical Microbiology

Abstract

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus responsible for several major outbreaks of disease in the past few decades. Infection of CHIKV triggers the formation of stress granules (SGs) in the host cytosol. SGs are RNA granules induced by global translational repression with G3BP1/2 as primary nucleators. During the late stage of infection, SGs are dispersed, giving rise to distinct *alpha-granules* that contain viral nsP3 and G3BP1/2 but lack other canonical SG components. However, the function and composition of the alpha-granules remain mainly enigmatic. Using a FACS-based method, we isolated the alpha-granules from CHIKV-infected U2OS cell lysates. Quantitative mass spectrometry identified alpha-granule protein components consistent with previous immunofluorescence and proximity-labeling studies. Notably, translation initiation factors and ribosomal proteins were not enriched, challenging previous suggestions that alpha-granules promote viral translation. Instead, a substantial fraction of alpha-granule-associated proteins are linked to the actin cytoskeleton, and smFISH revealed that genomic viral RNA is sequestered within the alpha-granules. Our findings suggest a potential role of alpha-granules in CHIKV genome protection and trafficking toward the plasma membrane for virion assembly. Furthermore, our FACS-based strategy provides a robust tool for dissecting the composition of biomolecular condensates.

Developing Methods to Detect Host-Viral Interactions During Chikungunya Virus Infection

Maxine Cañelas, Jared Bard

Texas A&M University, College Station, United States

Poster G-23, Graduate Medical Microbiology

Abstract

Protein and RNA interactions are vital for cellular processes, especially during viral infection in which RNA-binding proteins (RBPs) play key roles that are often contradictory, switching from antiviral to proviral functions. G3BP1 is a major human protein that is involved during infection by interacting with viral RNA (vRNA) and acting as a nucleator of stress granules (SG's), which are sites of translationally stalled mRNA and other RBPs. For the positive-sense single-stranded RNA alphaviruses like Chikungunya (CHIKV), G3BP1 has been shown to not only suppress viral RNA translation, but is also surprisingly necessary for efficient replication. A known interactor of G3BP1 is the nsP3 viral protein. Through this interaction, α-granules are formed, which are similar to SG's in composition, but have an unknown function. While many G3BP1 interactors have been identified, it is still unclear how CHIKV viral infection reshapes the full G3BP1 interactome, transforming its function to benefit viral replication. To study key interactions during infection, we will look at both the protein and RNA interactome of G3BP1 and, conversely, identify proteins interacting with CHIKV vRNA at multiple time points to determine their role in regulating viral infection. Currently, we are optimizing our labeling protocols, but the next step will be to further analyze the interactors through mass spec and RNA-Seq.

Undergraduate
General Microbiology

Mutations Cause a Differential Inhibition Effect on the Autophosphorylation of VraS Sensor Histidine Kinase

Ava Nguyen, Dana Al-Manaseer, May Abdel Aziz

University of Texas at Tyler, Tyler, United States

Poster UG-1, Undergraduate General Microbiology

Background: VraS is a membrane-bound histidine kinase in *Staphylococcus aureus*. As part of the VraSR two- component system, VraS acts as a sensor that detects cell wall stress, and undergoes autophosphorylation and then phosphorylates its associated response regulator, VraR, which activates a series of gene expression alterations that assist *S. aureus* in adapting to and surviving antibiotic treatment. Mutations in the VraS kinase domain have been found in antibiotic-resistant *S. aureus strains*, however the implications of these mutations on VraS inhibition have yet to be explored. In this work, we examined the inhibition profile of NH125, a compound that was reported to inhibit VraS activity in vitro and in culture, against the enzyme's wild-type and mutant forms.

Methods: The WT and five VraS mutants: T331I, E276K, M192I, S329L, and the D242G/G/L114S double mutant were expressed and purified from BL21 bacterial expression host. A coupled kinase assay was used to measure the kinetics of autophosphorylation of the enzymes. Dose-response curves were built to assess the half-maximal inhibitory concentration (IC50) of NH125 on the purified variants.

Results: Most of the mutations led to alteration of NH125-mediated inhibition of autophosphorylation. While the T331I mutation enhanced the inhibition from 41 μM for the WT VraS to 17 μM, the E276K mutation increased the IC50 to >500 μM.

Conclusion: NH125 exhibits a differential inhibitory effect on VraS variants, with most antibiotic-resistance mutations attenuating the inhibition. The data highlights kinase mutations in bacterial systems as key determinants that impact inhibitor efficacy and the design of resistance-modifying agents.

Understanding the Impact of Cell Motility on Cell Growth Behavior

Keemia Aria[1], Camilo Rodriguez Cortes[2], Matthew Bennett[2]

[1]Wharton County Junior College, Sugar Land, United States. [2]Rice University, Houston, United States

Poster UG-2, Undergraduate General Microbiology

Abstract

Signal propagation is inherently tied to gene expression, cell growth, and division. When studying signal propagation in complex environments, it is often assumed that cells are distributed uniformly across the media. However, it remains unclear how motility, an important factor in spatial organization, impacts the distribution of cells across complex environments. To investigate how the loss of motility affects growth patterns in media such as agar or carboxymethyl cellulose (CMC), we employed *Escherichia coli* CY027, a derivative of the E. coli strain BW25113, which is known for its low motility, and as a motile cell line, *E. coli* MG1655. First, we set up an experiment to test the difference in motility between strains using a motility assay. Growth behavior was tested quantitatively via growth curve and CFU counts, and qualitatively using microfluidic chips to visualize spatial organization in CMC and agar. Growth curves and CFU assays did not show statistically significant differences in generation time or viability between strains; however, microfluidic chip assays showed structural differences. In agreement with 1, previous research, *E. coli* CY027 formed cable-like structures in CMC, and now we show they exhibit the same behavior in agar, suggesting altered spatial patterning in the presence of polymers and absence of motility. These results emphasize the role of motility in bacterial growth behavior in complex environments, which may influence how bacteria coordinate functions like signaling, pathogenesis, and biofilm production in natural environments.

Spontaneous Mutations for Antibiotic Resistance in *Escherichia coli*

Brent Orji, Julian Ramirez, Christian Buford, Natalie Zarate, Wei-Chin Ho

University of Texas at Tyler, Tyler, United States

Poster UG-3, Undergraduate General Microbiology

Abstract

Microbial evolution is a key driver of antibiotic resistance, yet the mechanisms by which spontaneous mutations arise and contribute to this process remain poorly understood. These random genetic changes generate diversity within bacterial populations and may be influenced by environmental conditions, complicating efforts to predict microbial adaptation. Typically, resistance evolves through a combination of mutation, natural selection, and genetic drift, but the relative contribution of each factor is unclear, making it difficult to anticipate the pace and direction of resistance development. Here, we isolate the role of spontaneous mutation by repeatedly streaking single colonies of *Escherichia coli*, a method that amplifies genetic drift and minimizes selective pressure. This approach allowed us to examine how mutation alone contributes to resistance evolution. We cultured *E. coli* for over 1,000 generations on nutrient-rich media, both with and without the antibiotics ampicillin and streptomycin, and assessed resistance using minimum inhibitory concentration (MIC) assays. Our results show that *E. coli* populations evolved increased resistance to streptomycin, but not to ampicillin, indicating that spontaneous mutations more readily produce resistance-conferring changes for streptomycin. These findings suggest that the mutational supply for resistance is antibiotic-specific and shaped by the molecular targets and mechanisms of action of each drug. This work enhances our understanding of genotype-environment interactions and provides insight into how mutation-driven processes shape microbial evolution and the emergence of antibiotic resistance.

Phenotypic Diversity of *Escherichia coli* in North & East Texas

Christian Buford, Wei-Chin Ho

University of Texas at Tyler, Tyler, United States

Poster UG-4, Undergraduate General Microbiology

Abstract

Escherichia coli can be found in both the external environment and the intestines of most livestock mammals and poultry. Although it typically supports gut health, some strains can be harmful, affecting livestock, wildlife, and humans. Understanding how benign and harmful strains vary across seasons and spread through water bodies is important for wildlife managers, farmers, and ranchers. This research aims to study *E. coli* diversity in North and East Texas with seven sample sites. We isolated single colonies by identifying green/purple colonies on eosin-methylene blue agar. Antibiotic resistance phenotyping was then performed on these isolates by disc diffusion assay for six antibiotic treatments (Penicillin, Streptomycin, Erythromycin, Tetracycline, Nalidixic Acid, and Sulfamethoxazole-trimethoprim). Compared to the wild-type strain , all seven winter isolates are susceptible to erythromycin and penicillin. Compared to the wild-type strain in the lab, five out of seven spring isolates are susceptible to erythromycin and penicillin. In addition, one isolate is resistant to nalidixic acid. Compared to the wild-type strain in the lab, five out of six spring isolates are susceptible to erythromycin; four out of six summer isolates are susceptible to penicillin. In addition, one isolate is resistant to both nalidixic acid and streptomycin. Overall, we did not find any isolates resistant to tetracycline and sulpha/trimeth, suggesting that they are generally more effective treatments. Isolates in summer tend to show more cases of resistance compared to other seasons. The finding provides insight into environmental antimicrobial resistance for clinical applications.

The Effects of Historical Adaptation of Microbes in New Stressful Environments

Ivan Mendez, Kingsley Amoateng, Sora Kim, Julian Ramirez, Natalie Zarate, Wei-Chin Ho

The University of Texas at Tyler, Tyler, United States

Poster UG-5, Undergraduate General Microbiology

Abstract

Adaptation is a fundamental principle in evolutionary biology, as it allows organisms to develop traits that may affect their survival and reproduction in specific environments. Adaptive mutations often improve an organism's fitness in one environment, but when changing environments, the effects of the mutations may change. The historical effects of adaptive mutations are largely unknown, but they substantially determine the evolutionary dynamics in the early adaptation to complex environments. Moreover, if previously acquired mutations retain their advantages, organisms may transition more easily into new environments. However, if these mutations are detrimental in different environments, they may inhibit survival and adaptation. To explore this question, we examined three distinct bacterial populations evolved in (LB) medium for 900-days. The populations accumulated mutations that improved their fitness within this specific environment. To determine whether these acquired mutations were beneficial, neutral, or harmful in new environments, we transferred the adapted microbes into four new stressful environments: LB broth with alcohol, LB broth with MgSO4, LB broth with HCl, and Glucose minimal medium. The results showed an idiosyncratic nature of historical adaptation, an unpredictable pattern of fitness effects. In three of the 12 cases studied, historical mutations provided significant fitness advantages in new environments, suggesting some mutations are useful. However, in five cases, beneficial mutations became stressful, indicating that microbes thriving in LB broth struggled in the new environment. Moving forward, we plan to examine the mutations in more detail to determine the genetic basis of these population-specific differences.

Digital PCR for Pathogen Detection in Recreational Waters

Margaret Robison[1,2], Caroly Leija[2], Kristin Nemec[1], Nathan Elrod[1], Stephanie Brady[3], Janice Speshock[1], Jeff Brady[2]

[1]Tarleton State University, Stephenville, United States. [2]A&M AgriLife Research, Stephenville, United States. [3]Texas Institute for Applied Environmental Research, Stephenville, United States

Poster UG-6, Undergraduate General Microbiology

Abstract

Water testing for microbial health risks on Texas beaches employs EPA-validated culture-based detection of *Enterococcus* species. The current tests take more than 24 hours and provide no direct evidence of pathogenic microbe detection. Using a digital PCR instrument recently purchased by Tarleton State University and Texas A&M AgriLife Research, we developed a multiplexed fecal microbe and pathogen detection assay that provides rapid and highly informative results. Not only does the novel assay detect *Enterococcus* species, it also detects and quantifies several human pathogens to directly assess microbial health risks from recreational Texas beaches. Comparison of the novel PCR assay to the established culture-based assay suggests that future assessments of microbial health risks may be improved if culture-based detection is superseded by nucleic acid-based detection.

Influence of Interactions Between *C. jejuni* and *E. faecalis* on Environmental Stress Tolerance and Antimicrobial Resistance

Marwa Hemri, Marbella Canaca, Esmeralda Mendoza Lara, Todd Primm, Anand Karki

Sam Houston State University, Huntsville, United States

Poster UG-7, Undergraduate General Microbiology

Abstract

Campylobacter jejuni is a leading cause of foodborne illness worldwide and is commonly detected in retail poultry, where it frequently coexists with *Enterococcus* species. *Enterococcus* species are clinically challenging due to their high levels of antimicrobial resistance. Previous studies suggest that polymicrobial interactions can enhance *Campylobacter*'s tolerance to environmental stress, but their impact on antimicrobial resistance remains unclear. This study explores the influence of *C. jejuni* and *E. faecalis* coculture on survival under environmental challenges, such as low pH and biofilm formation, while also investigating their antibiotic resistance. We hypothesize that co-culture may promote resilience under stress conditions that are not evident in single-species cultures. Our results show that polymicrobial interaction influences survival of *C. jejuni* and *E. faecalis* during stress conditions highlighting the importance of considering microbial interactions when assessing survival and resistance of foodborne pathogens.

A Functional Genomic Dissection of Redox Stress Responses in *Candida albicans* Mutants

Reagan Chastain[1], Pedro Miramon Martinez[2], Michael Lorenz[2]

[1]LeTourneau University, Longview, United States. [2]UT Health McGovern Medical School, Houston, United States

Poster UG-8, Undergraduate General Microbiology

Abstract

Candida albicans is a commensal yeast colonizing 50% of the population but under predisposing conditions can cause infections, with mortality rates near 40%. A key defense is macrophage phagocytosis, which *C. albicans* evades by filamentous hyphae formation, enabling escape and dissemination. RNA-seq profiling during macrophage confrontation showed that 48% of upregulated genes are uncharacterized. We hypothesized systematic deletion, and phenotypic screening will uncover novel regulators of stress adaptation and immune evasion. We focused on C1_11950W (putatively *PXP2*), previously identified as connected to oxidative stress resistance. Using SC5314 as the genetic background, CRISPR-Cas9 was utilized to generate homozygous *pxp2Δ/Δ* mutants, strains 1935 and 1936. Survival assays quantified by propidium iodide uptake showed strain 1935 failed to filament or escape macrophages and remained within acidic phagosomes shown by pHrodo green labeling. LDH release assays indicated reduced macrophage damage, and XTT-based fungal biomass quantification confirmed enhanced clearance of the mutant. Whole genome sequencing revealed both mutants retained at least one copy of *PXP2*, with strain 1935 displaying a high SNP burden and deletions, suggesting off-target effects. Despite this, 1935 exhibited a stable, relevant phenotype in macrophage interactions. Bone marrow derived macrophages confirmed reduced hyphal formation, macrophage damage, and fungal survival in 1935. These findings underscore the utility of macrophage-based functional screens for revealing phenotypes not apparent *in vitro*. Although genetically complex, strain 1935 shows a robust defect in immune evasion and survival, warranting transcriptional and genetic dissection. Future RNA-seq will clarify whether *PXP2* or a linked locus underlies this niche specific attenuation.

Cultivability and Potential Antibiotic Resistance of Bacteria in Probiotic Drinks

Rene Hernandez Martinez, Emily Yu, Alan Lizarraga, Riqing Yu

University of Texas at Tyler, Tyler, United States

Poster UG-9, Undergraduate General Microbiology

Abstract

Probiotic drink supplements are a growing source of beneficial microbes to human health. In this study, a total of 49 bacterial strains have been successfully isolated from varied commercial drink brands under both anaerobic and aerobic conditions. The isolated strains include 8 strains from *Lacticaseibacillus rhamnosus*, 5 strains from *Streptococcus thermophilus*, 4 strains from *Lacticaseibacillus casei*, 1 strain from *Lacticaseibacillus paracasei*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*. Amongst these, *Bifidobacterium* species are the most used probiotic bacterial strains due to their important roles maintaining gut homeostasis, enhancing immune function, and contributing to overall health. Commercial powdered dietary supplements frequently include *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis.*Limited research has been reported on the survivability and cultivability of *Bifidobacterium* on the shelf after industrial processing. The study was to isolate and culture strains from commercially labeled probiotic products under strict anaerobic conditions and aerobic conditions, demonstrating the presence and viability of bacteria within probiotic products. The observed growth characteristics under anaerobic conditions strongly support the successful recovery of these four strains. This work provides a foundation survey of the microbial diversity present in commercial studies aimed at assessing their antibiotic resistance and other health risks and evaluating their probiotic efficacy. The antibiotic resistance and related health risk study are undergoing by using PCR detection, gene sequencing, and antibiotic drug bioassays for the four *Bifidobacteria* and other relevant probiotic species in probiotic drinks widely available in the current market.

Tracking *Salmonella* in Retail Chicken Liver: A Genomic Perspective

Marcos A. Montelongo, Layla R. Behrens, Madison M. Schultz, Todd P. Primm, Anand B. Karki

Sam Houston State University, Huntsville, United States

Poster UG-10, Undergraduate General Microbiology

Abstract

Salmonella remains a major contributor to foodborne illnesses, hospitalizations, and deaths in the USA and worldwide. Consumption of contaminated retail poultry products is the primary route of transmission, and chicken liver–associated outbreaks of salmonellosis have been reported. Meanwhile, the rise of multidrug-resistant *Salmonella* is a growing public health concern. In our recent study, 31% of retail chicken liver samples collected from grocery stores in Walker County, TX were found to be contaminated with *Salmonella* spp. In this study, we performed comparative genomic analysis of twelve selected *Salmonella enterica* strains using long-read whole genome sequencing. Most isolates belonged to the Enteritidis and Kentucky serovars, which are commonly associated with human *Salmonella* outbreaks, and phylogenetic analysis using NDTree and MLST/cgMLST grouped the isolates into three distinct clades. SPIFinder analysis indicated that some pathogenicity islands were unique among the isolates and may have been acquired through past horizontal gene transfer events. Antimicrobial resistance genes and virulence factors were largely similar across all samples, with minor variations. In conclusion, the high prevalence of *Salmonella* in retail chicken liver, along with the presence of virulence and antimicrobial resistance genes that may contribute to human illness, remains a significant food safety concern.

Undergraduate
Medical Microbiology

Battle of the Brushes: A Comparative Study on Oral Bacterial Impact Between Miswak and Traditional Toothbrush Methodology

Senna Al-Hashmi, Raina Williams, Sin Man Mak, Megan Romeo

Dallas College, Dallas, United States

Poster UG-11, Undergraduate Medical Microbiology

Abstract

Recognized as one of the earliest toothbrushes, Miswak has been widely studied for its oral health benefits. Sourced from various trees, its natural fibers help to remove plaque while offering inherent antimicrobial properties. As a biodegradable and naturally sourced alternative, Miswak reduces environmental impact while promoting cost effective and more antimicrobial properties than the traditional toothbrush. In this study, we examined the bacterial profiles and quantities in the oral cavity before and after using two different types of Miswak compared to conventional nylon toothbrush and fluoride toothpaste. Saliva samples were collected before and after use of Miswak and traditional toothbrush followed by taking colony counts of the overall oral bacteria and oral streptococci. In the participants studied, results showed a reduction in colony-forming units for oral streptococci after use of Miswak and variable results for overall oral bacterial count. Overall, Miswak demonstrated a greater reduction in colony-forming units in oral streptococci after five consecutive days of use compared to traditional toothbrushes. This suggests that Miswak offers enhanced antibacterial benefits, reinforcing its potential as an effective oral hygiene tool.

Contribution of ssaA to USA300 *Staphylococcus aureus* Virulence in Defensin-Deficient Mice

Matthew Thomas[1], Bhavani Balasundarasekar[1], Monica Thanigaivel[1], Rebecca Keogh[2], Jacob Hogins[1], Alexander Horswill[2], Xintong Dong[1]

[1]The University of Texas at Dallas, Richardson, United States. [2]University of Colorado Anschutz Medical Campus, Aurora, United States

Poster UG-12, Undergraduate Medical Microbiology

Abstract

Staphylococcus aureus (SA), a Gram-positive bacterium, is responsible for a wide range of skin infections, from impetigo to abscesses. The rise of methicillin-resistant S. aureus (MRSA) has increased infection rates, mortality, and public health concerns, underscoring the need to better understand host-pathogen interactions that shape infection outcomes. Our lab performed RNAseq analysis comparing wild-type and defensin-deficient (Def cKO) mice intradermally infected by MRSA and revealed broad upregulation of virulence factors, with ssaA identified as one of the most strongly up-regulated genes. Antimicrobial peptides (AMPs), particularly defensins, represent a critical component of the skin's innate immunity, acting directly to kill bacteria and indirectly by promoting neutrophil activation through the Mrgpra2 receptor. To investigate the role of ssaA in this context, we infected WT, Defensin Knockout (Def cKO) and Mrgpra2-deficient (Mrgpra2 dKO) mice with either WT USA300 or ΔssaA USA300 strains. Bacterial burden was quantified by colony-forming unit (CFU) analysis at the site of infection 24 hours post-infection. Our results show that the ΔssaA mutants exhibited higher bacterial loads in WT mice compared to Def cKO mice. These findings suggest that ssaA is an important virulence determinant under AMP-mediated immune pressure and provide mechanistic insight into how S. aureus adapts to innate defenses.

The Role of Symbiotic Status of *Exaiptasia diaphana* in *Serratia marcescens* Pathogenesis

Amy Joffrion, Tanya Brown

University of Texas at Tyler, Tyler, United States

Poster UG-13, Undergraduate Medical Microbiology

Abstract

Symbiosis does not just shape how reef organisms live; it may also shape how they die. As a model for coral-algal symbiosis, *Exaiptasia diaphana* offers a window into how mutualism can influence disease proliferation. *Serratia marcescens* is a bacterium that functions as an opportunistic pathogen in cnidarians. It is also proven to cause mortality in sea anemone *Exaiptasia diaphana*. Under stressful conditions *E.diaphana* can lose their Symbiodinium which leads to decreased photosynthetic energy, slowed growth, and limited reproduction. We investigated whether symbiotic state impacts lethality of *S. marcescens* infections. We conducted 10-day bacterial challenges at 30C to assess mortality at three concentrations (10^6, 10^7, 10^8 CFU/mL). As a comparison we also conducted 10-day challenges using bacteria isolated from *E.diaphana.* The bacteria from *E.diaphana* showed no mortality over all microbial concentrations. At 10^8 CFU/mL *S. marcescens* showed 100% mortality by day four in symbiotic anemones while there was complete mortality by day three in aposymbiotic anemones at the same concentration. Lower bacterial concentrations of *S. marcescens* showed no mortality over the 10-day microbial challenge. Symbiotic state may play an important role in *S. marcescens* lethality with aposymbiotic anemones dying earlier than symbiotic *E.diaphana*. Symbiodinium not only provide important nutritional support for symbiotic cnidarians but also aid in defending against pathogens.

CRISPR Interference in a Hypervirulent *Streptococcus agalactiae* MLST-17 Strain

Ben Klemp[1], William Cutts[1], Gideon Hillebrand[2], Thomas Hooven[2,3,4], Brandon Kim[1]

[1]University of Texas at Dallas, Richardson, United States. [2]University of Pittsburgh School of Medicine, Pittsburgh, United States. [3]Richard King Mellon Institute of Pediatric Medicine, Pittsburgh, United States. [4]UPMC Children's Hospital of Pittsburgh, Pittsburgh, United States

Poster UG-14, Undergraduate Medical Microbiology

Abstract

Group B *Streptococcus* (*Streptococcus agalactiae* , GBS) is a leading cause of neonatal meningitis. Typically, GBS is a commensal organism colonizing the gastrointestinal and urogenital tracts; however, it can cause opportunistic infections. The strain COH1, serotype III, multi-locus sequence type 17 (MLST-17), expresses a variety of potent virulence factors. Understanding of the genes contributing to pathogenesis is limited due to the difficulty of altering the COH1 genome making classical mutagenesis challenging. We have hijacked the endogenous bacterial CRISPR-Cas9 system to produce effective knockdowns of genes in a process referred to as CRISPR interference (CRISPRi). A catalytically deadened version of Cas9 (dCas9) has been generated that has retained its ability to bind DNA at targeted sites but lost its endonuclease activity. Expressing a guide RNA targeting a gene of interest in this Cas9 strain reduces transcription. We have validated the CRISPRi system in COH1 by manipulating the expression of the toxin encoding cyl operon and the regulator of this operon, CovR. The expected hemolysis phenotype is observed following knockdown. When cyl is targeted, we observe less hemolysis and when CovR is targeted, we observe increased hemolysis. CRISPRi offers a tunable, targeted alteration of gene expression, allowing study of genes that may otherwise be difficult to modify. Recently we have generated a library of single guide RNAs (sgRNA) to every protein coding gene within COH1. This library is a tool to facilitate the study of phenotypic changes within GBS and may contribute to the generation of a complete knockdown library in the future.

Mutations in the Sensor VraS Histidine Kinase Alter the Virulence of *Staphylococcus aureus*

Reagan Shofner, Liaqat Ali, Salima Karki, Sana Petkar, Thanya Chaisuwan, May Abdelaziz

The Department of Pharmaceutical Sciences and Health Outcomes, Fisch College of Pharmacy, The University of Texas at Tyler, Tyler, United States

Poster UG-15, Undergraduate Medical Microbiology

Abstract

Staphylococcus (S.) aureus is a highly pathogenic organism that can cause lethal human infections, mainly in the skin and respiratory tract. *S. aureus* utilizes the VraSR two-component system to regulate cell wall synthesis and facilitate resistance against cell wall-targeting antibiotics. Mutations in VraS were detected in some resistant clinical isolates, but the causative link between mutation and resistance remains unclear. We have investigated the effect of several VraS mutations on *S. aureus* persistence and virulence compared to a wild-type Newman strain susceptible to antibiotics.

Methods: Eight single-point mutations (I5N, G9V, L114S, M192I, D242G, A314T, S329L, T331I) and one double mutation (L114S/D242G) were introduced to the *S. aureus* Newman strain genome using recombineering techniques coupled with CRISPR-Cas9 counterselection. Minimum inhibitory concentrations (MIC) of three chemically diverse antibiotics were assessed. Biofilm formation was measured by a colorimetric assay using crystal violet staining. Lysostaphin lysis curves were monitored using OD_{600} measurements. Gene expression levels of the resistance-associated *blaZ* gene, which translates to the β-lactamase enzyme, were determined by qRT-PCR.

Results: The mutants showed a 2 – 16-fold increase in MIC for the tested antibiotics and equal or increased resistance to lysostaphin. While the biofilm formation was decreased in all mutants compared to the WT Newman strain, the expression levels of *blaZ* were significantly increased in most mutants.

Conclusion: VraS mutations cause enhanced antibiotic resistance in *S. aureus* with altered virulence that may impact host-pathogen interactions.

Role of DltA in *Staphylococcus aureus* Skin Infection Under Defensin Deficiency

Monica Thanigaivel Thanigaivel[1], Bhavani Balasundarasekar[1], Matthew Thomas[1], Rebecca Keogh[2], Jacob Hogins[1], Alexander Horswill[2], Xintong Dong[1]

[1]The University of Texas at Dallas, Richardson, United States. [2]University of Colorado Anschutz Medical Campus, Aurora, United States

Poster UG-16, Undergraduate Medical Microbiology

Abstract

Staphylococcus aureus (SA) is a major pathogen causing skin and soft tissue infections as well as invasive diseases such as endocarditis and sepsis. Using an intradermal infection model, we performed RNAseq analysis comparing WT and a defensin cluster knockout (Def cKO) mouse skin post SA infection. Our transcriptomic analysis revealed strong upregulation of multiple virulence factors, with DltA showing one of the highest elevations in defensin cluster knockout (Def cKO) mice. *dltA* modifies the bacterial cell wall to resist cationic antimicrobial peptides (AMPs) and contributes to antibiotic tolerance, making it a key candidate for understanding host-pathogen interactions. To test this, we compared wild-type USA300 and Δ*dltA* strains in WT and Def cKO mice and quantified bacterial burden 24 hours post-infection. In WT mice, Δ*dltA* mutants persisted at levels similar to WT bacteria. In Def cKO mice, however, Δ*dltA* mutants were cleared more efficiently than WT bacteria, indicating that defensins specifically shape the fitness advantage conferred by *dltA*. These findings establish *dltA* as both a virulence determinant and a modulator of bacterial survival under defensin pressure, offering mechanistic insight into the role of the *dltA* system in interacting with host AMPs.

Composition and Characterization of Gingival and Molar Bacteria and Potential Pathogenic Relationships with Oral Diseases

Khanh Huyen Cao, Emily Yu, Alan Lizarraga, Ri-Qing Yu

Department of Biology at UT Tyler, Tyler, United States

Poster UG-17, Undergraduate Medical Microbiology

Abstract

The diversity and characteristics of gingival and molar associated bacteria directly contribute to periodontal diseases, oral diseases, and systemic effects on the human body. This study identifies bacterial strains presenting on students' gumline and teeth using 16S rRNA gene sequencing and analyzing their association with oral health. Bacterial strains were collected, isolated, and purified from 150 students' gumline and teeth. Genomic DNA extraction, PCR amplifications and sequencing of 16S rRNA genes were performed to identify the bacterial strains with BLAST in the NCBI database. Our preliminary results show that 29 different species in the gums and 33 species in the teeth of students were recorded. Within the gingival tissues, the dominant species included *Rothia dentocariosa* (7 strains), *Streptococcus salivarius* (6), and *Nesseria sp.* (6), while the most abundant species in teeth were *R. dentocariosa* (19 strains), *S. salivarius* (6), *Staphylococcus epidermidis* (5) and *Staphylococcus aureus* (5), and *Neisseria subflava* (5). While *Rothia dentocariosa* is an opportunistic pathogen that relates to oral diseases, *Streptococcus salivarius* and *Nesseria sp.* are commensal bacteria that are commonly found in healthy oral microbiomes. Other strains of bacteria were also detected and analyzed as a part of this research. The result indicates the complex oral microbiome composition and interaction as well as providing the foundation for oral health preventative approach.

Defensins in Urogenital Epithelial Defense Against Uropathogenic *E. coli*

Mikaila Labit, Maliha Batool, Xintong Dong

The University of Texas at Dallas, Richardson, United States

Poster UG-18, Undergraduate Medical Microbiology

Abstract

Recurrent urinary tract infection (rUTI) is primarily caused by uropathogenic *Escherichia coli* (UPEC), which persists in the bladder and may be sustained by reservoirs in the vagina. Emerging evidence suggests that interactions between vaginal and bladder microbiota, including Gardnerella, influence UPEC adhesion and invasion; however, the epithelial immune mechanisms governing these processes remain poorly understood. Defensins, antimicrobial peptides secreted by epithelial cells, are critical components of innate defense, yet their regulation and function across the bladder-vagina axis are not well defined. To address this gap, we are conducting both *in-vivo* and *in-vitro* studies. Here, we focus on in vitro approaches to investigate defensin expression in immortalized human bladder (5637) and vaginal (VK2) epithelial cells following infection with symptomatic UPEC (UTI-89) or an asymptomatic bacteriuria (ASB) strain, compared with uninfected controls. In parallel, we will assess the functional contribution of defensins to bladder epithelial cell (BEC) immunity using primary BEC cultures derived from wild-type C57BL/6 and defensin conditional knockout mice, as well as CRISPR/Cas9-engineered 5637 human BECs lacking the defensin gene cluster. UPEC entry, intracellular survival, and host cell apoptosis will be quantified, and rescue experiments will be performed by reintroducing specific defensins. We anticipate that defensin-deficient BEC cultures will exhibit increased extracellular and intracellular bacterial burdens compared to wild-type cells, with larger and more numerous intracellular bacterial communities (IBCs) and greater epithelial cell death. These approaches will reveal how defensins regulate epithelial responses to UPEC and influence rUTI pathogenesis.

Measuring Caspase Activation and Apoptosis in Calu-3 Cells Upon Exposure to Pneumolysin

Jackson Elliott, Joshua Tadegegn, Ali Azghani

University of Texas at Tyler, Tyler, United States

Poster UG-19, Undergraduate Medical Microbiology

Abstract

Streptococcus pneumoniae (*sp*) is a Gram-positive bacterium that commonly colonizes the upper respiratory tract. While often asymptomatic, *sp* can cause severe complications such as pneumonia and meningitis in children, the elderly, and immunocompromised populations. The pathogenesis of *sp* is largely mediated by pneumolysin (PLY), a 53-kDa cholesterol-dependent cytolysin. In lung epithelial cells, PLY promotes programmed cell death, such as apoptosis and pyroptosis. However, the exact mechanism and its dependence on time and dose remain unclear. Previous data collected by our lab show that treatment of Calu-3 cells with a 5 µg/mL PLY induced expression of pro-inflammatory cytokines IL-1β and IL-18. We hypothesize that exposure of Calu-3 lung epithelial cells to PLY will result in increased activation of caspases 3/7 and caspase 1, alongside LDH release. We also expect that co-treatment with a PLY-neutralizing antibody will decrease pore-forming activity, though cytotoxic effects may still be present due to PLY Interacting with other components of the host cell. To test our hypothesis, Calu-3 cells will be cultured in a 96-well culture plate to confluence and treated with 5, 10, or 15 µg/mL PLY. Caspase activity will be quantified using FAM-FLICA caspase activity assays, and membrane damage will be assessed by an LDH release assay. To assess antibody effects, cells will be co-treated with PLY and anti-PLY-4. Antibody amounts will be determined by evaluating the IC_{90} of anti-PLY-4 at each toxin concentration using an LDH assay. Caspase activity and LDH release will be measured at 5, 10, and 24 hours post-treatment.

Impact of 5-LO inhibitor, Zileuton, on the efficacy of antifungal therapy against Cryptococcus in vitro

Khoi Nguyen, Natalia Castro Lopez, Floyd Wormley

Texas Christian University, Fort Worth, United States

Poster UG-20, Undergraduate Medical Microbiology

Abstract

Individuals with severe immunocompromised conditions face a 10% to 25% mortality rate when infected with *Cryptococcus* fungi. To better understand Cryptococcus pathogenesis and explore strategies to mitigate infection severity, research has identified leukotrienes (LTs) as critical lipid modulators exploited by fungi. LTs are derived from arachidonic acid through the enzymatic activity of 5-lipoxygenase (5-LO). Studies in our lab have shown that mice deficient in 5-LO exhibit reduced disease severity when exposed to *Cryptococcus* deneoformans strain 52D. In contrast, wild-type (WT) mice infected with the same strain developed meningoencephalitis, leading to a higher mortality rate. These findings suggest modulating host 5-LO activity could be a therapeutic strategy for reducing *Cryptococcus* infection severity. Our lab demonstrated that treating WT mice with zileuton, a 5-LO inhibitor, increased survival rates. To further evaluate the potential of 5-LO inhibitors like zileuton in alleviating brain-related symptoms during infection, this project examines the drug interaction between zileuton and commonly used antifungal treatments—amphotericin B, 5-flucytosine, and fluconazole—to determine any impact on antifungal efficacy. Our preliminary results indicate that zileuton does not interfere with fluconazole and amphotericin B activity, suggesting zileuton could be used with antifungal drugs to ameliorate the symptoms during Cryptococcus. Next, we are evaluating the effect of zileuton when two antifungal are used in combinations.

Evaluation of vaccine-mediated immune response against Cryptococcus neoformans

Christine Sayegh, Natalia Castro Lopez, Floyd Wormley Jr.

Texas Christian University, Fort Worth, United States

Poster UG-21, Undergraduate Medical Microbiology

Abstract

Cryptococcus neoformans is a pathogenic fungus that can cause cryptococcosis, affecting the lungs and central nervous system with potentially morbid consequences. This pathogen is particularly aggressive in individuals with impaired T-cell function, such as those with AIDS or on immunosuppressive medications. There are currently no vaccines available for this pathogen, and a limited arsenal of antifungals is available. Our lab has developed a C. neoformans strain that produces mouse IFN-ɣ, called H99ɣ, that induces protective immunity against subsequent infection with wild-type C. neoformans in mouse models of cryptococcosis. We aim to use variants of this strain by knocking out genes previously known to induce protection in mice, to create a robust vaccine candidate, and better understand the immune response against Cryptococcus and develop new therapies. In this study, our goal is to evaluate the efficacy of various newly developed C. neoformans vaccine mutants to induce protective immune responses against C. neoformans. These strains are exposed to monocytes and examined for their ability to upregulate MHC-II and initiate protective immune responses. RNA will be isolated from tissues extracted from mice immunized with the different C. neoformans strains: H99ɣ, LW10, LW10ɣ, sre1ΔLW10ɣ, and sgl1ΔLW10ɣ; the mRNA transcripts of immune cells responding to subsequent infection with C. neoformans evaluated. By using the information derived from these transcripts, we aim to identify key determinants of protection against cryptococcosis. Using the transcriptomic data, we can determine the best candidate to further evaluate for its capacity to elicit protective immune responses in immunocompromised hosts.

Staff
General Microbiology

Mycobacterium abscessus Biofilms Promoted on Plastic Pipes Compared to Metal Pipes

Kristin Dean, Rachel Wilsey, Adrian Hornby, Jennifer Honda

University of Texas Health Science Center at Tyler, Tyler, United States

Poster SP-1, Staff General Microbiology

Abstract

Nontuberculous mycobacteria (NTM) form biofilms within household plumbing, particularly showerheads and sink faucets. NTM within these biofilms are sloughed off in aerosolized water droplets, inhaled, and cause NTM pulmonary disease in susceptible individuals. Prior studies demonstrate that clinical NTM isolates readily form biofilms on plastic surfaces compared to metal surfaces. However, biofilm formation on household plumbing has not been characterized for environmental NTM isolates. We hypothesize reduced biofilm formation on metal pipes compared to plastic using a well-water-derived *Mycobacterium abscessus* (MABS) isolate. Sterile 2” metal (copper, stainless steel) and plastic (CPVC, PEX) pipe sections were submerged in 100ml Middlebrook 7H9 broth and inoculated with 105 CFU/ml MABS. At 48 and 96hrs post-inoculation, pipes were sonicated and CFU tabulated. Additionally, 0.5”x0.5” pieces of each pipe material were incubated in 10ml of MABS culture for 48 and 96hrs, AFB stained and visualized under high-power microscopy. MABS recovery was shown to be significantly lower in metal pipes compared to plastic pipes ($p<0.0001$), highlighting the preference of MABS to grow in plastic piping. In fact, plastic CPVC showed the highest MABS recovery of the piping materials at a comparable level to the 7H9 control. These results demonstrate the preference of environmental MABS to grow in plastic piping systems compared to metal. Further work to mimic environmental growth conditions using tap water, a biofilm reactor, and expansion to other MABS isolates is ongoing.

Mycobacterium abscessus from Hawai'i Highlight Significant Morphological and Molecular Complexity Among Smooth-Rough Environmental Isolates

Rachel Wilsey[1], Michael Teve[1], Adrian Hornby[1], Kaelyn Pacpaco[2], Kristin Dean[1], Lianghao Ding[1], Chelsea Raulerson[1], Ethan Hill[2], Yvonne Chan[2], Jennifer Honda[1]

[1]University of Texas Health Science Center at Tyler, Tyler, United States. [2]'Iolani School, Honolulu, United States

Poster SP-2, Staff General Microbiology

Abstract

Mycobacterium abscessus (MABS) is an environmental pulmonary pathogen known to exist in both smooth and rough morphotypes. However, why these two forms exist and the incipient cause(s) for morphological change remain unknown. It's surmised smooth is the environmental form that initiates lung infection, but transitions to the rough, more virulent morphotype during disease. We hypothesize morphotypes are driven by genetic and molecular modifications to the cell wall that confer environmental and host survival advantages to smooth and rough isolates respectively. Of 139 MABS environmental isolates from Hawai'i, 91% (n=126) were smooth and 1% (n=2) were rough, confirming the environmental dominance of smooths. The remaining 11 (8%) showed mixed morphotypes, which were separately isolated. High-resolution imaging mosaics revealed colony circularity decreased in roughs compared to smooths (mean decrease of 0.082 [$p<0.0001$]). Analysis of whole genome sequencing data indicated that smooth-rough pairs cluster by pair instead of morphotype, suggesting that minor genomic differences within pairs may influence morphotype transitions instead of broad morphotype-specific differences. Additionally, *de novo* Nanopore assemblies exposed amino acid mutations and indels within the *rmlA* gene that could impact cell wall formation. Phylogenetic analysis revealed 54% of isolates clustered with DCC1 using the current cgMLST scheme and RAxML, with isolates from both morphotypes found in DCC1 and non-DCC1. RNAseq identified multiple differentially expressed genes between morphotypes, including upregulation of a flotillin family protein gene (membrane fluidity) and down-regulation of the *mps2* (GPL-associated) gene in roughs. Together, these results highlight that MABS morphotype biology is inherently complex and multifaceted.

Modular Genome Engineering Platform to Study G3BP1 Function in Human Cells

Zhaohan Xu, Jared Bard

Texas A&M, College Station, United States

Poster SP-3, Staff General Microbiology

Abstract

Many viruses depend on essential host proteins. Direct genetic deletions of these proteins are often impossible, but chemical-genetic tools let us control protein levels and test function in living cells. For instance, G3BP1 (Ras GTPase-activating protein-binding protein 1) is a multifunctional RNA-binding protein involved in antiviral responses, stress granule assembly, and cancer progression. While G3BP1 is not essential, its deletion causes homeostatic compensation which makes it difficult to determine its normal function. To accurately dissect its structure-function relationship we sought to develop a conditional depletion of endogenous G3BP1 while stably expressing exogenous mutant variants. To achieve this, first, we install an evolved Bxb1 (eeBxb1) landing pad at the AAVS1 safe harbor using prime editing. This enables site-specific integration of exogenous G3BP1 variants. Next, we make endogenous G3BP1 depletable by tagging it with a mini-AID (mAID) via CRISPR–Cas9 and expressing TIR1 by piggyBac transposon system. We also knock out G3BP2 by CRISPR–Cas9 to remove redundancy. With this platform, we can not only manipulate endogenous G3BP1 protein levels to study its role in stress granule dynamics, antiviral defense, and cancer, but also adapt this modular framework to investigate other proteins with redundant paralogs.

Disruption of Native β-Lactamases in *Acinetobacter baumannii* Affects Multiple Physiological Pathways

Christine Pybus, Charles Birt, Nitika Kuve, Weston McNish, Joseph Boll

UT Dallas, Richardson, United States

Poster SP-4, Staff General Microbiology

Abstract

The β-lactam class of antibiotics includes some of the safest and most widely prescribed antimicrobials that can treat a broad array of infections. However, widespread resistance to them has emerged. Resistance mechanisms including extended-spectrum β-lactamases (ESBL) and carbapenemases (CRE), are increasingly prevalent in clinical isolates and are associated with poor outcomes for patients. β-lactam antibiotics bind to penicillin-binding proteins (PBPs) and inhibit transpeptidase activity and peptidoglycan biosynthesis, which ultimately leads to bacterial cell death. One strategy bacteria use to avert this outcome is the production of β-lactamases, enzymes that cleave the β-lactam ring in β-lactam antibiotics to inhibit antimicrobial activity. *Acinetobacter baumannii* is an important global pathogen that not only acquires horizontally transferred β-lactamases but inherently carries them on the chromosome. Our laboratory is interested in characterizing the functions of these intrinsic enzymes, not only from an antibiotic resistance standpoint, but in consideration of their evolution from PBPs and possible involvement in other physiological processes. To this end, we have disrupted the native *oxa51* and *ampC* genetic loci in *A. baumannii* strain 17978 and 19606, as well as utilized the Manoil transposon library in multi-drug-resistant *A. baumannii* strain 5075_UW to obtain disruptions in *oxa51*, *ampC* and *oxa23*. We characterized the antibiotic sensitivity, growth kinetics, potential suppressors, cellular morphology, and genetic expression under various growth conditions for several of these strains. Taken together, these studies suggest that *A. baumannii* intrinsic β-lactamase expression may be regulated and that the levels of expression are important for cell viability.

Transition Metals and Hawai'i Nontuberculous Mycobacteria: Magnified Analyses at the Water Tap

Adrian Hornby[1], Ettie Lipner[2], Rachel Wilsey[1], Riley Johnson[3], Stephen Nelson[4], Greg Carling[5], Jennifer Honda[1]

[1]UT Tyler Health Science Center, Tyler, United States. [2]NIAID, National Institutes of Health, Bethesda, United States. [3]UT Tyler School of Medicine, Tyler, United States. [4]University of Utah, Salt Lake City, United States. [5]Brigham Young University, Provo, United States

Poster SP-5, Staff General Microbiology

Rationale: Transition metals in groundwater, in particular vanadium (V) and molybdenum, have been associated with nontuberculous mycobacteria (NTM) pulmonary disease (PD). Domestic plumbing fixtures harbor viable NTM and are presumed to be important point sources for NTM acquisition. However, the role of transition metals has not been investigated in tapwater. We hypothesize that tapwater transition metals correlate with NTMPD prevalence compared to groundwater and influence NTM pathogenicity. We focus our study on Hawai'i, an NTMPD hotspot.

Methods: In total, 391 water samples were collected from faucets on O'ahu, Maui and Hawai'i Island and measured for selected transition metals, plus Al, As, Sb and Pb, using ICP-MS. Matching water biofilm swabs were sampled from the same faucets from which water was collected, cultured for viable NTM, and suspected NTM species were identified species *rpoB* gene sequencing.

Results: Tapwater with the highest V concentrations corresponded to aquifers that were previously identified as higher *Mycobacterium avium* complex (MAC) PD risk areas. NTM were isolated most frequently from taps on Hawai'i Island, and MAC and *Mycobacterium abscessus* were the most commonly isolated species of respiratory significance. NTM-positive taps were associated with significantly higher copper and nickel concentrations. In matched taps, hot water was associated with significantly higher Mn, Ni and Co.

Conclusion: For tapwater, correlations between V and NTMPD are observed at fine resolution from high-density Hawai'i data. Metals such as Cu, Ni, and Co within water systems should be further investigated for their role in metabolism, pathogenicity and/or influence on NTM growth.

Epetraborole in the Wild West of Mycobacterium

Sanjay Singh[1], Gunavanthi D Boorgula[1], Rodolfo-Amaro Galvez[2], Megan Devine[2], Tawanda Gumbo[3], Shashikant Srivastava[1,4]

[1]Division of Infectious Diseases, Department of Medicine, University of Texas at Tyler School of Medicine, Tyler, United States. [2]Department of Medicine, Section of Pulmonary and Critical Care, University of Texas at Tyler School of Medicine, Tyler, United States. [3]IMPI Biotech Consortium Inc., Tateguru, Zimbabwe. [4]Department of Cellular and Molecular Biology, The University of Texas Health Science Centre at Tyler, Tyler, United States

Poster SP-6, Staff General Microbiology

Background: Treatment of pulmonary disease caused by *Mycobacterium tuberculosis* (*Mtb*) and non-tuberculous mycobacteria, namely, *M. avium* complex (MAC), *M. abscessus* (Mab), and *M. kansasii* (Mkn), still remains a challenge. We tested epetraborole, an oral inhibitor of bacterial leucyl-tRNA synthetase, for activity against Mtb and NTMs.

Methods: Reference strains of *Mtb*-H37Rv (ATCC# 27294), MAC (ATCC# 700898), Mab (ATCC# 19977), and Mkn (ATCC# 12478), and libraries of clinical isolates were used in the minimum inhibitory concentration (MIC) and static concentration-response studies to determine the MIC distribution, efficacy, and potency of epetraborole against pathogenic mycobacteria.

Results: The lowest MIC_{50} (0.25 mg/L) and MIC_{90} (0.5 mg/L) of epetraborole were for 59 clinical isolates of Mab. We observed a bimodal epetraborole MIC distribution for *Mtb* (n=21), MAC (n=50), and Mkn (n=20) clinical isolates. Epetraborole maximal microbial kill (E_{max} or efficacy in log_{10}CFU/mL) against MDR-*Mtb* was 3.21, for Mab, MAC, and Mkn was 2.45, 3.85, and 2.52, respectively. The concentration required for 50% of E_{max} (EC_{50} or potency in mg/L) for *Mtb* was 0.24, for Mab, MAC, and Mkn was 0.19, 0.28, and 54, respectively.

Conclusion: The two major findings were – (1) bimodal epetraborole MIC distribution suggests the possibility of pre-existing epetraborole-resistant clones of the aforementioned pathogens with reduced susceptibility, and (2) the epetraborole efficacy and potency against *Mtb*, and NTMs suggest that while the new sheriff in town is effective, it needs the help of other drugs to control these maniacs to prevent emergence of drug resistance during the therapy.

Sulbactam/Durlobactam-Based Double β-lactam-β-lactamase Combinations to Treat *Mycobacterium abscessus* Pulmonary Disease.

Avneesh Shrivastava[1], Gunavanthi D Boorgula[1], Sanjay Singh[1], Danaleigh Stiles[2], Pamela J McShane[3], Megan Devine[4], Tawanda Gumbo[5], Shashikant Srivastava[6]

[1]Division of Infectious Diseases, Department of Medicine, University of Texas at Tyler School of Medicine, Tyler, United States. [2]Undergraduate Medical Education, School of Medicine, University of Texas at Tyler, Tyler, United States. [3]Critical Care and Pulmonary Branch, National Heart, Blood, and Lung Institute, National Institute of Health, Bethesda, United States. [4]Section of Pulmonary and Critical Care, Department of Medicine, School of Medicine, University of Texas at Tyler, Tyler, United States. [5]IMPI Biotechnology Company Incorporated, Mount Hampden, Zimbabwe. [6]Department of Cellular and Molecular Biology, The University of Texas Health Science Center at Tyler, Tyler, United States

Poster SP-7, Staff General Microbiology

Background: β-lactams are prone to hydrolysis by *Mycobacterium abscessus* (MAB) β-lactamase enzymes. There is some evidence that dual β-lactam therapy could restore susceptibility, but ideal pairing is unknown. We tested sulbactam/durlobactam (Sul/Dur) with nine antibiotics as dual β-lactam-β-lactamase combinations to treat MAB pulmonary disease (MAB-PD).

Methods: We performed (i) minimum inhibitory concentration (MIC) experiments using reference strain (ATCC#19977), (ii) MIC distribution using 63 clinical isolates, and (iii) static concentration-response studies with amoxycillin, cefdinir, ceftriaxone, cefalexin, cefuroxime, imipenem, meropenem, tebipenem, and faropenem. Experiments were performed in triplicate.

Result: MIC of Sul/Dur for the ATCC#19977 strain was 4 mg/L; the concentration used in subsequent combination studies. There was 64-fold (range: 19 to 105) reduction in individual drug MIC among the 63 clinical isolates; highest for ceftriaxone (105-fold), and lowest for cefdinir (19-fold). The ranking of drugs based on kill below stasis (as mean±standard Error $\log_{10}$ CFU/mL) was 5.36±0.24 for imipenem and meropenem, 4.79±0.68 for tebipenem, 4.41±1.87 for cefdinir, 3.92±0.27 for faropenem, 3.68±0.22 for cefuroxime, 2.840.26 for cefalexin, 2.35±0.33 for ceftriaxone, and 2.28±0.28 for amoxicillin.

Conclusion: Sul/Dur improved MIC and kill activity of β-lactams against MAB. Given the observed changes in MIC and kill effect, we propose to advance ceftriaxone and imipenem to test with Sul/Dur as dual β-lactam-β-lactamase backbones, followed by the addition of other active drugs to create multidrug combinations for effective treatment of MAB-PD.

Interkingdom Interactions Between Environmental Rapid Growing Mycobacteria and *Acanthamoeba* That Drive Virulence

Madelyn Terrell[1], Jonathan Awaya[2], Ronnie Mooney[3], Ernest Mui[4], Fiona Henriquez-Mui[3], Jennifer Honda[1]

[1]University of Texas Health Science Center at Tyler, Tyler, United States. [2]University of Hawai'i at Hilo, Hilo, United States. [3]University of Strathclyde, Glasgow, United Kingdom. [4]University of the West of Scotland, Blantyre, United Kingdom

Poster SP-8, Staff General Microbiology

Abstract

The environment's role in the emergence of infectious disease is an acknowledged area of scientific importance. Yet, the interkingdom interactions between environmental microbes influencing pathogen emergence remains to be elucidated. A widely interrogated interkingdom interaction is the dynamic prey *i.e*., bacteria and predator *i.e.,* free-living amoebae (FLA) relationship. In the context of NTM and *Acanthamoeba*, the opposite may be true; that is, it's hypothesized that NTM survival within *Acanthamoeba* promotes NTM virulence and infecting of human macrophages. We screened 34 international environmental FLA samples and found 85% (n=26) were positive for *Acanthamoeba* spp. Furthermore, 91% (n=31) of the 34 FLA samples were positive for intracellular NTM by *rpoB* PCR, suggesting NTM and *Acanthamoeba* interact on a global scale. Moreover, among 547 soils from Hawai'i, 187 (34%) were NTM culture positive. So far, viable *Acanthamoeba* have been isolated from five soil samples. To interrogate whether nonpathogenic amoeba-adapted rapid growing NTM emerge more virulent for human cells, *A. lenticulata* ATCC #30481 was infected with *Mycobacterium neoaurum* (MNEO, MOI 1:1) for 168 hours and amoeba-adapted isolates used to infect THP1 macrophages. A clinical isolate of MNEO displayed decreased CFU upon infection into macrophages after incubation in *A. lenticulata* compared to an environmental isolate ($p = 0.0002$) Taken together, 168HR adaptation of MNEO in *A. lenticulata* does not increase NTM virulence. Future experiments include infection assays using matched soil amoeba-NTM pairs. Elucidating how natural microbial ecosystems and microbial interactions influence infectious disease dynamics provides valuable insight on how to improve risk management.

Fellow
Medical Microbiology

Model-Informed Precision Dosing of Sulbactam-Durlobactam for the Treatment of *Mycobacterium abscessus* Pulmonary Disease

Sanjay Singh[1], Avneesh Shrivastava[1], Gunavanthi D Boorgula[1], Mary C Long[2], Brian Robbins[2], Pamela J McShane[3], Tawanda Gumbo[4], Shashikant Srivastava[1,5]

[1]School of Medicine, University of Texas at Tyler, Tyler, United States. [2]Advanta Genetics, Tyler, United States. [3]Critical Care and Pulmonary Branch, National Heart, Blood, and Lung Institute, National Institute of Health, Bethesda, United States. [4]IMPI Biotech Consortium Inc., Tateguru, United States. [5]Department of Cellular and Molecular Biology, University of Texas Health Science Center at Tyler, Tyler, United States

Poster FP-1, Fellow Medical Microbiology

Background. New drugs and regimens are needed to improve the therapeutic outcomes of the *Mycobacterium abscessus* pulmonary disease (MAD-PD). We performed preclinical pharmacokinetics/pharmacodynamics (PK/PD) studies paired with computer-aided clinical trial simulations to determine the clinical dose of sulbactam/durlobactam (Sul/Dur) for MAB-PD.

Methods. First, we performed a combined dose-response and dose-fractionation study in the hollow fiber model system of MAB (HFS-MAB) using the reference ATCC#19977 strain, mimicking intrapulmonary pharmacokinetics of 10 different human equivalent doses over 14 days. Second, Monte-Carlo simulation (MCE) experiments were performed, accounting for different creatinine clearance scenarios, to determine the probability of target attainment (PTA) using 480,000 virtual patients.

Results. Sul/Dur MIC of the MAB reference strain was 4 mg/L. In the HFS-MAB, Sul/Dur microbial kill and antimicrobial resistance were linked to % time concentration persists above MIC ($\%T_{MIC}$), with target exposure of $50\%T_{MIC}$. Sulbactam-durlobactam killed 3.85 $\log_{10}$CFU/mL below stasis with regrowth by day 14. Renal function and the dosing schedule were determined as the important drivers for achievement of >90% PTA for sulbactam, where the sulbactam PTA improved as renal dysfunction worsened. For example, MCE predicted that with creatinine clearance < 30 ml/min, a 1G q24h sulbactam dose would be optimal.

Conclusion. The combination of preclinical drug development tools we used highlights the therapeutic potential of Sul/Dur for the treatment of MAB-PD. Further, studies are warranted combining Sul/Dur with other effective drugs to develop novel therapeutic regimens for MAB-PD.

A Global Regulator Reshapes the Host Cell Transcriptional Landscape to Modulate Phage Lysis Timing

Guadalupe Valencia-Toxqui, Jolene Ramsey

Department of Biology, Center for Phage Technology, Texas A&M University, College Station, United States

Poster FP-2, Fellow Medical Microbiology

Abstract

The time it takes for a phage to induce the rupture of a host cell and release new viral particles is referred to as lysis timing. This can be controlled by proteins known as holins, which form micron-scale holes in the inner membrane of the bacterial cell. For instance, the Mu phage possesses a lysis cassette that includes an endolysin (gp22), spanins (gp23/gp23a), a hypothetical protein (gp24), and releasin (gp25) instead of a traditional holin. The primary aim of this study is to elucidate the function of Gp24 in Mu phage. Deletion of gp24 in Mu phage revealed that it is a non-essential gene, while its overexpression affects β-galactosidase production and reduces swimming motility in *Escherichia coli*. HHpred analysis revealed a 96% probability of structural similarity between Gp24 and the global regulator DksA of *E. coli*. We observed that Gp24 plays a regulatory role in lysis, as Mu lysogens overexpressing this protein exhibited delayed cell lysis. To investigate this further, we evaluated lysis using two plasmids: one containing three different versions of the lysis cassette of Mu phage (from gp22 to gp25) and another with only gp24. In all combinations tested, Gp24 delayed lysis timing. Given these results, we measured Gp25 levels in the constructs mentioned above following Gp24 expression and found that Gp25 levels increased after Gp24 expression. Based on these observations, we propose a model in which Gp24 influences lysis timing by positively modulating the expression of Gp25 in Mu phage and negatively regulating promoters in *E. coli*.

Microbiome Networks Reveal Structural Differences in Response to Soil Tillage and Soil Spatial Dynamics

Omy Ogbughalu[1], Richard Griffin[1], Olukayode Kuloyo[2], Nicolas Tsesmetzis[2], Ram Ray[1], Javad Barouei[1]

[1]Prairie View A&M University, Prairie View, United States. [2]Shell Technology Centre, Houston, United States

Poster FP-3, Fellow Medical Microbiology

Abstract

Soil health is crucial for plant growth and food production. Soil microbial communities regulate plant growth through nutrient and biogeochemical cycling. These communities are influenced by plant type, soil properties, agricultural activities, environmental, and other anthropogenic factors. This study aimed to analyze soil microbial community structure and soil microbial intra- and inter-actions. The study aimed to understand the impact of intrinsic factors (pH, salinity, electrical conductivity), extrinsic factors (cropland vs. pastureland), and spatial variations (15cm, 30cm, 60cm depth) on microbial community structure in East Central Texas. Targeted amplicon sequencing of 16S rRNA and ITS2 genes, were used to analyze and obtain soil-microbe interactions and to map the underlying microbial association networks. Thermoleophilia and Verrucomicrobiae were more prevalent in both cropland and pastureland. However, Actinobacteria was more prominent in cropland, while Bacilli was more abundant in pastureland. Agaricomycetes dominated fungal communities in both cropland and pastureland. Key members contributing to microbial structural changes were identified using multivariate statistical analyses. Results revealed that pH, electrical conductivity, and depth significantly influenced soil-microbe interactions. Microbial co-occurrence network results identified correlations and hub taxa specific to individual soil environments, indicating targeted responses in microbe-microbe interactions as a function of their immediate environment. Network statistical tests revealed more stable microbial interactions in pastureland soils compared to cropland. This study revealed that microbial dominance/abundance alone does not necessarily reflect involvement in nutrient and biogeochemical cycling. These new insights advance our understanding of soil microbial ecology and interactions, informing management strategies for resilient and sustainable agroecosystems.

M cells and WISP Cells: Specialized Epithelial Players Shaping Human Airway Mucosal Immunity

Samuel Alvarez-Arguedas[1], Khadijah Mazhar[2,3], Andi Wangzhou[2,3], Ishwarya Ishwarya Sankaranarayanan[2,3], Gabriela Gaona[1], John T. Lafin[4], Ron B. Mitchell[5], Theodore J. Price[2,3], Michael U. Shiloh[1,6]

[1]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, United States. [2]School of Behavioral and Brain Sciences, University of Texas at Dallas, Richardson, United States. [3]Center for Advanced Pain Studies, University of Texas at Dallas, Richardson, United States. [4]Department of Urology, University Texas Southwestern Medical Center, Dallas, United States. [5]Department of Otolaryngology, University of Texas Southwestern Medical Center, Dallas, United States. [6]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, United States

Poster FP-4, Fellow Medical Microbiology

Abstract

Airway mucosa is the initial site of encounter for airborne pathogens such as *Mycobacterium tuberculosis*, influenza virus, and SARS-CoV-2. Mucosa-associated lymphoid tissues (MALT) orchestrate early immune responses by sampling antigens through specialized epithelial cells, including microfold (M) cells. While intestinal M cells have been extensively characterized, their airway counterparts remain poorly understood, limiting our knowledge of how pathogens interact with this barrier. Using single-nucleus RNA sequencing, we generated a comprehensive atlas of the human adenoid and identified 26 distinct epithelial and immune cell types. Trajectory analysis revealed that airway M cells develop from club cell progenitors and display a transcriptional program distinct from intestinal M cells, underscoring tissue-specific strategies of antigen uptake. In addition, we identified a previously unrecognized epithelial population that we named WISP (*Waldeyer's ring Interferon-Stimulated Population*) cells, enriched for interferon-stimulated genes. These cells likely represent a dedicated antiviral defense population at the airway mucosal barrier. Together, our findings provide the first detailed characterization of human airway M cells and reveal WISP cells as a novel epithelial player in mucosal immunity. By defining how these specialized cell types contribute to antigen sampling and innate defense, this work advances our understanding of early host–pathogen interactions in the airway and establishes a foundation for mucosal vaccine development.

Spatial Transcriptomic Analysis of HIV and Tuberculosis Coinfection in a Humanized Mouse Model Reveals Unique Transcription Patterns, Immune Responses and Early Morphological Alterations

Sitaramaraju Adduri, Jose Alejandro Bohorquez, Rajesh Mani, Omoyeni Adejare, Joshua Kleam, Mounika Duggineni, Andy Omeje, Torry Tucker, Nagarjun Konduru, Guohua Yi

The University of Texas Health Science Center at Tyler, Tyler, United States

Poster FP-5, Fellow Medical Microbiology

Abstract

Mycobacterium tuberculosis (*Mtb*) and human immunodeficiency virus (HIV) coinfection is one of the biggest public health concerns worldwide. Both pathogens are adept at modulating immune response and, in the case of *Mtb*, even inducing structural modification of the affected tissue. The present study aimed at understanding the early phenotypical and functional changes in immune cell infiltration in the affected organ, using a humanized mouse model. The humanized mice were infected with either HIV or *Mtb* in single infection, or with both pathogens in coinfection. Three weeks after the infection, lung samples were collected, and spatial transcriptomics analysis was performed. This analysis revealed high infiltration of $CD4^+$ T cells in *Mtb* infection, but not in HIV or coinfection. Coinfected mice also showed a minimal number of NK cells compared to the other groups. In addition to infection status, histological features also influenced the immune cell infiltration pattern in the lungs. Two distinct airway regions with distinct immune cell abundance patterns were detected by spatial transcriptome profiling. A lymphoid cell aggregate detected in coinfection lung exhibited distinct transcript profile. The cellular architecture in the lymphoid cell aggregate did not follow the spatial patterns seen in mature granulomas. However, lymphoid cell aggregates exhibited granuloma gene expression signatures, and pathways associated with reactive oxygen species production, oxidative phosphorylation, and TGFβ and interferon signaling similar to granulomas. This study revealed specific transcription patterns, immune responses and morphological alteration signaling in the early stage of HIV and *Mtb* infections.

Production of High Binding Affinity Nanobodies against Viral (VZV) Protein, Using Phage Display Technology

Arnaud Kombe

University of Texas Southwestern Medical Center, Dallas, United States. University of Science and Technology of China, Hefei, China

Poster FP-6, Fellow Medical Microbiology

Abstract

Neurotropic viruses like varicella-zoster virus (VZV) still remain a challenge for public health because of their ability to hide inside neuronal cells and escape from host immunity, making them difficult pathogens to clear once infected. Although considered among mild symptom diseases, VZV-related diseases are highly morbid, with severe complications, including mental development deficits, meningoencephalitis, and postinfectious encephalopathy (in varicella cases) and vasculitis, zoster sine herpete, and postherpetic neuralgia, after reactivation. Currently available VZV diagnostics, vaccines, and treatment, including VZV-antibodies, though effective in prevention and healing symptoms, cannot eradicate VZV. An alternative treatment approach, use of nanobodies, is promising for catching-up VZV inside neuronal cells. Thus, this talk aims to present, the phage display method, an efficient process to develop nanobodies and share the nanobodies produced from related works, with high binding affinity to VZV protein and that can be used as potent neutralizers.