

# 2026 FALL MEETING

Microbes Make Our World

HOUSTON, TEXAS | October 15 - 17, 2026



## ABSTRACT BOOK

BioScience Research Collaborative | 6500 Main Street, Houston, TX

Hosted by

 **UTHealth Houston**  
**McGovern Medical School**

Microbiology and  
Molecular Genetics

# Contents

|                                                                                               |     |
|-----------------------------------------------------------------------------------------------|-----|
| <b>Speaker Abstracts</b> .....                                                                | 2   |
| General Microbiology Session 1: “Microbial Survival Strategies” .....                         | 3   |
| General Microbiology Session 2: “Microbial Ecology, Microbiomes, and Evolution (MEME)” .....  | 9   |
| Medical Microbiology Session 1: “Microbial adaptation to host environment” .....              | 15  |
| Medical Microbiology Session 2: “Pathogenesis and Host Defense” .....                         | 21  |
| Scientific Plenary Session: “Decoding Microbiology Systems: Antibiotics, Phages and AI” ..... | 27  |
| <b>Poster Abstracts</b> .....                                                                 | 33  |
| General Microbiology Undergraduate Student Posters .....                                      | 34  |
| Environmental Microbiology Undergraduate Student Posters.....                                 | 45  |
| Medical Microbiology Undergraduate Student Posters .....                                      | 53  |
| General Microbiology Graduate Student Posters .....                                           | 67  |
| Environmental Microbiology Graduate Student Posters.....                                      | 80  |
| Antimicrobial Strategies Graduate Student Posters .....                                       | 90  |
| Genomics Graduate Student Posters.....                                                        | 102 |
| Virology Graduate Student Posters .....                                                       | 111 |
| Medical Microbiology Group 1 Graduate Student Posters .....                                   | 123 |
| Medical Microbiology Group 2 Graduate Student Posters .....                                   | 136 |
| Early Career Fellows Posters .....                                                            | 151 |
| Researchers and Faculty Posters .....                                                         | 164 |
| <b>Presenter Index</b> .....                                                                  | 181 |

# Speaker Abstracts

## **General Microbiology Session 1: “Microbial Survival Strategies”**

# Development of a Fluorescent Based Transcriptional Assay for Analyzing the Effects of Fidaxomicin Resistance Mutations in *Clostridioides difficile* RNA Polymerase

Wei Zhao<sup>1</sup>, Xinyun Cao<sup>2</sup>, Julian G. Hurdle<sup>1</sup>

<sup>1</sup>Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, Department of Translational Medical Sciences, Texas A&M Health Science Center, Houston, United States. <sup>2</sup>Department of Microbiology, UT Southwestern Medical Center, Dallas, United States

## Abstract

**Background:** *Clostridioides difficile* infection (CDI) has been listed as an urgent threat to public health worldwide. About 25% of CDI patients experience recurrent disease (rCDI), and 45-65% of these go on to have multiple bouts of rCDI. Fidaxomicin (FDX), a narrow-spectrum macrolide, selectively targets the  $\beta$  and  $\beta'$  subunits of *C. difficile* RNA polymerase (RNAP). Nevertheless, mutations in the  $\beta$  and  $\beta'$  subunits of RNAP have been identified in FDX-resistant (FDX-R) strains and are associated with clinical treatment failure. However, it is unknown how these FDX-R mutations affect the transcriptional efficiency of RNAP. **Hypothesis:** A fluorescence-based assay enabling real-time quantification of RNAP activity will reveal how FDX-R mutations impact transcription efficiency in *C. difficile*. **Method:** *C. difficile* RNAP holoenzyme and sigma factor A were expressed in *E. coli* and purified. We established a fluorescence aptamer-based transcription assay using a DFHBI-1T binding to the RNA aptamer encoded in vector pTwist under control of a *C. difficile* rRNA promoter. **Results:** We observed that transcription was dependent on the enzyme, NTPs, and the circular DNA template. Transcriptional activity was inhibited by rifampicin and fidaxomicin, respectively. Analysis of site-directed RNAP mutants is ongoing to examine if mutations affect rates of transcription and response to fidaxomicin inhibition. **Conclusion:** This real-time fluorescence platform provides a powerful approach for elucidating how RNAP mutations alter transcription efficiency and drug susceptibility, offering a valuable tool for evaluating fidaxomicin resistance at the molecular target level.

## Lipopolysaccharide remodeling as a resistance mechanism to cell envelope targeting antibiotics

Ella Stanley<sup>1</sup>, Teresa Sullivan<sup>2</sup>, Anna Konovalova<sup>1,2</sup>

<sup>1</sup> UTHealth Houston, Houston, United States. <sup>2</sup> The University of Texas MD Anderson Cancer Center  
UTHealth Houston Graduate School of Biomedical Sciences, Houston, United States

### Abstract

The rise of antibiotic resistance in Gram-negative pathogens presents a major global health crisis. This resistance is largely driven by the impermeable nature of the bacterial outer membrane (OM). The  $\beta$ -Barrel Assembly Machinery (BAM) complex, specifically the essential, surface-exposed component BamA, is a promising target for novel therapeutics. The BamA inhibitor MRL-494 effectively disrupts OM protein assembly, leading to OM permeability and cell death. The Konovalova lab isolated *Escherichia coli* mutants resistant to MRL-494 targeting PmrAB and LpxM. Our goal is to understand how OM remodeling confers resistance to cell-envelope targeting inhibitors. To determine whether cell-envelope perturbations directly activate the PmrAB two-component system, we treated strains expressing an mScarlet reporter under control of PmrAB-regulated *eptA* and *arn* promoters with various antibiotics and monitored fluorescence in a time-course experiment. Antibiotics that influence lipopolysaccharide (LPS) levels caused a dose-dependent increase in fluorescence, suggesting that PmrAB supports LPS homeostasis. To investigate how LPS structural alterations drive MRL-494 resistance, we generated *lpxM* and *lpxL* knockouts, which both exhibited increased minimum inhibitory concentrations (MICs). SDS-PAGE and Western blot analyses revealed that LPS levels in these mutants remain stable despite decreased OM proteins upon MRL-494 treatment. These findings indicate that OM remodeling, specifically LPS modifications, confers resistance to envelope-targeting inhibitors by promoting LPS biosynthesis. Further experiments can be conducted to understand the underlying molecular mechanisms and translational implications for other envelope-targeting inhibitors.

## Genomic diversity of *Streptococcus gallolyticus* subsp. *gallolyticus*, its type VII secretion system, and an expanded LXG toxin landscape is revealed via comparative genomic analysis

Guadalupe Calderon, Jyoti Tamang, Sarah Woodfin, Isaac Prah, Julian Hurdle, Yi Xu

Texas A&M Health Science Center Institute of Biosciences and Technology, Houston, United States

### Abstract

*Streptococcus gallolyticus* subsp. *gallolyticus* (Sgg) is an opportunistic pathobiont associated with bloodstream infections, infective endocarditis, and colorectal cancer, particularly in older adults and immunocompromised patients. Although Sgg is increasingly recognized for its clinical importance, the genomic diversity of this species and the distribution of key virulence-associated systems, including the type VII secretion system (T7SS), remain incompletely understood. Here, we performed a comparative genomic investigation of 76 Sgg isolates originating from a range of host species and geographic locations. Phylogenetic analysis, multilocus sequence typing, and characterization of the core and pan-genomes revealed several prevalent sequence types (STs), some of which showed significant associations with geographic location or source of isolation. Examination of the T7SS genomic region identified five previously undescribed organizational subtypes and demonstrated that T7SS architecture was strongly associated with ST lineage. Genome-wide screening also markedly broadened the catalog of T7SS-associated LXG domain-containing polymorphic toxins (LXG toxins) in Sgg and revealed substantial strain-level differences in toxin repertoires. Phenotypic analysis further showed that biofilm-forming capacity differed significantly among isolates carrying distinct T7SS subtypes. Together, these results highlight the genomic and lineage-associated diversity of Sgg and demonstrate extensive variation in both T7SS organization and LXG toxin content. The observed relationship between T7SS subtype and biofilm formation additionally points to a possible contribution of this secretion system to biofilm-related phenotypes. Further experimental studies are needed to establish whether T7SS variation directly influences biofilm formation and to define the functions of the newly identified LXG toxins.

## Investigating the Role of the Nonsense Mediated mRNA Decay Pathway (NMD) in Regulating the Cellular Response to Arsenic Stress in *Saccharomyces cerevisiae*.

Stephnie Nwaiwu, Bessie Kebaara, Bernd Zechmann

Baylor University, Waco, United States

### Abstract

Arsenic is a widespread environmental toxin that disrupts essential cellular processes, including energy production, protein synthesis, redox homeostasis, and genome stability.<sup>1–3</sup> Its different chemical forms interfere with cells in distinct ways: As(V) Arsenate can mimic phosphate, whereas As(III) Arsenite can bind critical protein thiols and disrupt protein function.<sup>1</sup> While yeast has well-characterized mechanisms for detoxifying arsenic,<sup>4</sup> how cells regulate their RNA during this stress remains poorly understood.

Nonsense-mediated mRNA decay (NMD) is a conserved, translation-dependent RNA surveillance and regulatory pathway mediated by Upf1p, Upf2p, and Upf3p.<sup>5, 6</sup> Beyond eliminating aberrant transcripts, NMD also regulates natural mRNAs<sup>5</sup> and has been linked to metal homeostasis.<sup>6</sup> To address this gap, we investigated whether NMD-mediated RNA regulation shapes cellular adaptation to arsenic stress.

Analysis of publicly available transcriptomic datasets identified *ARR1* and *ARR3*, key arsenic-response genes, as potential NMD-regulated transcripts. Growth assays further showed that NMD-deficient strains display altered arsenic tolerance, with *upf1Δ*, *upf2Δ*, and *upf3Δ* strains retaining growth under arsenic stress. To probe this relationship genetically, *UPF1* was deleted in strains lacking individual ARR pathway components. Notably, loss of *UPF1* partially suppressed the arsenic-sensitive phenotype of *arr3Δ*, suggesting that NMD may influence additional pathways that support arsenic tolerance when arsenite export is compromised. Together, these findings support a role for NMD in shaping arsenic stress adaptation and motivate ongoing studies of transcript stability, RNA modification, arsenic accumulation, and cellular ultrastructure.

## Exploring the cell wall with LPS-free *Acinetobacter*

Brent W. Simpson<sup>1</sup>, Amanda B. McLean<sup>2</sup>, and M. Stephen Trent<sup>2,3</sup>

<sup>1</sup> Biology Department, Texas A&M University, College Station, Texas, USA. <sup>2</sup> Department of Infectious Diseases, University of Georgia, Athens, Georgia, USA. <sup>3</sup> Department of Microbiology, University of Georgia, Athens, Georgia, USA.

### Abstract

Bacteria have evolved different cell compositions that provide advantages in their natural environments or as pathogens. Gram negatives have a three-layered cell envelope with an inner membrane, peptidoglycan, and outer membrane. The outer membrane has lipopolysaccharide (LPS) or lipooligosaccharide (LOS) only on the cell surface. LPS and LOS differ in the presence (LPS) or absence (LOS) of an O-antigen. LPS/LOS is essential in most Gram negatives providing a permeability barrier and rigidity to the cell. Peptidoglycan is a mesh of polysaccharide chains crosslinked with short peptides, that also provides cellular rigidity. The outer membrane and peptidoglycan are essential structures and major targets of antimicrobial therapy. The Gram-negative pathogen *Acinetobacter baumannii* normally produces an LOS-containing outer membrane but is remarkably able to survive even with complete loss of LOS. We have used comparison of *A. baumannii* with and without LOS as a critical tool to understand how it builds its cell envelope. A transposon sequencing approach in *A. baumannii* missing LOS unveiled new aspects about peptidoglycan degradation and cell division. Understanding the biogenesis of the outer membrane and peptidoglycan has broad implications in bacterial pathogenesis, antimicrobial resistance, and the development of new therapeutic strategies.

## **General Microbiology Session 2: “Microbial Ecology, Microbiomes, and Evolution (MEME)”**

## Adult gut microbiome perturbation and aggressive escalation in *Gryllobates sigillatus*

Christian Guerrero, Amanda Brown

Texas Tech University, Lubbock, United States

### Abstract

Gut microbes can influence animal behavior, but we know much less about how they shape social interactions in insects. In male crickets, aggressive encounters progress through a stereotyped sequence from antennal assessment to escalating physical combat, retreat, and the establishment of dominance. This sequence provides a useful system for asking whether internal physiological state changes how animals escalate conflict. Studies in *Drosophila* suggest that gut bacteria can affect aggression, but it is unclear whether the adult gut microbiome influences aggressive behavior in crickets. Here, we test whether changing the adult gut microbial state alters the progression of male–male fights in *Gryllobates sigillatus*. Adult males receive vehicle, a broad-spectrum four-antibiotic mixture, viable *Lactiplantibacillus plantarum* 299v, or heat-killed 299v. Each treated male fights once against a fight-naïve, vehicle-treated opponent. We record behavior from first antennal contact through escalation, retreat, and dominance, and quantify maximum escalation, transition time, and fight outcome with an automated behavioral-classification pipeline validated against human scoring. We also measure movement before contact to identify general treatment effects on activity. We preserve focal crickets immediately after each fight to characterize their gut microbial communities. Comparing vehicle and antibiotic treatments tests whether disruption of the resident microbiome alters aggression, whereas comparing live and heat-killed 299v tests whether any probiotic effect depends on bacterial viability. Initial analyses will include approximately 30 focal males per treatment. Together, this study tests whether the adult gut microbiome contributes to how crickets escalate and resolve social conflict.

## Ticks and Their Associated Pathogens in Domestic Dogs from Ecotones of the Peruvian Amazon Basin.

Oliver A. Bocanegra<sup>1,2</sup>, Cusi Ferradas<sup>1,2</sup>, Winnie M. Contreras<sup>1,2</sup>, Giuseppe Troncoso Palacio<sup>1</sup>, Amber Holley<sup>3</sup>, Katarina Gasic<sup>1</sup>, Veronica K. C. Perez<sup>2</sup>, Danny Perez<sup>2</sup>, Raul Flores Mara<sup>2</sup>, Andres G. Lescano<sup>2,4</sup>, Maureen Laroche<sup>1,4,5,6</sup>

<sup>1</sup>Department of Microbiology & Immunology, School of Medicine, University of Texas Medical Branch, Galveston, United States. <sup>2</sup>Emerge, Emerging Diseases and Climate Change Research Unit, Faculty of Science and Philosophy, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru. <sup>3</sup>Department of Experimental Pathology, School of Medicine, University of Texas Medical Branch, Galveston, United States. <sup>4</sup>Clima, Latin American Center of Excellence for Climate Change and Health, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru. <sup>5</sup>Department of Population Health & Health Disparities, School of Public and Population Health, Galveston, United States. <sup>6</sup>Paul G. Allen School for Global Health, College of Veterinary Medicine, Washington State University, Pullman, United States

### Abstract

In transition zones between distinct ecological settings, or ecotones, interactions between domestic dogs and wildlife may facilitate the introduction of tick species and their associated pathogens into domestic environments. In a cross-sectional study conducted between 2022 and 2024, ticks and epidemiological data were collected from dogs in ecotone areas of Madre de Dios, Southeastern Peru (Amazon Basin), across various communities we stratified as urban, mining, agricultural, and sylvatic, based on their infrastructure and economic activities. Ticks were identified morphologically and molecularly, prior to being screened by qPCR for multiple tick-borne agents, with qPCR-positive samples confirmed by Sanger sequencing. Prevalence ratios were estimated using bivariate Poisson regression with robust variance clustered at the household level. Among 376 dogs sampled, 133 (35.4%) were infested with ticks. Ticks were identified as *Rhipicephalus linnaei*, *R. microplus*, *Amblyomma oblongoguttatum*, and *A. ovale*, with highest species diversity in agricultural areas. Animal and zoonotic pathogens, including *Ehrlichia canis*, *Anaplasma platys*, *Rickettsia parkeri* strain Atlantic Rainforest, *Bartonella quintana*, *B. vinsonii* subsp. *berkhoffii* type III, *Borrelia* spp., and *Ehrlichia chaffeensis*-like were identified in our tick samples. Pathogen-positive ticks were more frequently found on dogs with flea co-infestation, field-working owners, and residences in agricultural or forest settings. This study reports the first molecular detection of *B. vinsonii* and *Borrelia* spp. in ticks from Peru, as well as unreported tick/pathogen associations. Further studies are needed to decipher the ecology of these agents in ecotones, but this study highlights the dynamic risk of exposure to various tick-borne pathogens in these areas.

# Tillage Practices Restructure Bacterial Communities in Soybean Rhizospheres Across Arkansas and Texas

Samuel Osei, Angelica Torres, Mohammad Kamruzzaman

University of Texas at Arlington, Arlington, United States

## Abstract

Microbial inoculants and conservation practices, including no-till and reduced tillage, are increasingly used to improve crop productivity and soil health, yet their effects can be mediated by resident microbial communities and local environmental conditions that vary among agricultural fields. Conventional tillage can disrupt soil structure, accelerate organic matter loss, and increase greenhouse gas emissions. We investigated the effects of different tillage practices (conventional vs. conservation tillage) and bacterial inoculation on rhizosphere microbiome responses in soybean fields across Arkansas and Texas. We characterized bacterial communities in the soybean rhizosphere using 16S rRNA gene amplicon sequencing under conventional tillage and either no-till or reduced-tillage practices with three inoculation treatments: a drought-tolerant *Bradyrhizobium* inoculant, a commercial inoculant, and an uninoculated control. Despite differences between the two field environments, tillage produced consistent community-level responses. Bray-Curtis, Jaccard, weighted UniFrac, and unweighted UniFrac analyses distinguished the two tillage systems in both states, whereas bacterial inoculation did not significantly alter overall community composition. Alpha-diversity responses differed between Arkansas and Texas, and the bacterial taxa associated with conservation versus conventional tillage were largely site-specific. Predicted nitrogen-cycling potential also shifted in response to different tillage practices, but the affected pathways and directions of these changes differed between the two states. Together, these results reveal a reproducible effect of conservation tillage on the assembly of soybean rhizosphere communities without convergence toward a universal microbial composition or functional profile. Thus, tillage management may act as a common ecological filter, while local environmental conditions determine which microorganisms and functions ultimately respond.

## Growth constraints dictate evolution of nafcillin resistance in MRSA

Namrata Bonne<sup>1</sup>, Satvik Kompella<sup>1</sup>, Nicholas Dillon<sup>1</sup>

<sup>1</sup> The University of Texas at Dallas, Richardson, United States

### Abstract

*Staphylococcus aureus* is a formidable pathogen with an impressively fast ability to evolve resistance. With the increasing prevalence of multidrug resistant methicillin-resistant *S. aureus* (MRSA), it is crucial to understand how resistance evolves. In this study, we explored the influence of fitness constraints on evolution of nafcillin resistance in MRSA using two experimental evolution strategies: the tolerance adaptive laboratory evolution (TALE), which maintains continuous exponential growth and the resistance adaptive laboratory evolution (RALE), which permits entry into the stationary phase. Our findings reveal remarkably divergent mechanisms. The TALE strains exhibited 128-fold nafcillin resistance and faster growth while maintaining their normal colony variant (NCV) phenotype and harboring known mutations associated with *mecA* upregulation. Conversely, the RALE strains achieved extremely high level of resistance (1024-fold) by switching to small colony variant (SCV) phenotype. These SCVs demonstrate the hallmark traits of persistence: slower growth and increased biofilm production. Whole genome sequencing revealed that the RALE strains lack traditional *mecA* regulatory mutations but rather harbor amplification of the SCCmec cassette, suggesting a simple but powerful way of bacterial resistance development. These SCVs are able to regain their lost fitness upon reversion to NCV phenotype, with the accompanied loss of the 1024-fold resistance and genomic amplifications. Ongoing experiments are investigating the translation of these complex evolutionary dynamics and resistance trajectories to the fluctuating host-imposed selective pressures *in vivo*. This work highlights the exceptional capacity of MRSA to use genome plasticity and phenotype switching to circumvent traditional regulatory pathways for survival under extreme antibiotic pressure.

## Microbial symbionts drive coral immunity across species

Lauren E. Fuess<sup>1</sup>, Erin N. Shilling<sup>1</sup>, Ashley Rossin<sup>2</sup>, Alex Veglia<sup>3</sup>, Grace Klinges<sup>4</sup>

<sup>1</sup>Department of Biology, Texas State University, San Marcos, Texas. <sup>2</sup>NOAA Atlantic Oceanographic and Meteorological Laboratory, Miami, Florida. <sup>3</sup>Department of Biology, University of Puerto Rico-Mayaguez, Mayaguez, Puerto Rico. <sup>4</sup>Island Conservation, Kailua-Kono, Hawaii

### Abstract

Reef-building corals are well known to be intimately dependent on numerous microbial partners, with microbes contributing significantly to physiological processes ranging including nutrient exchange and cycling. Increasing evidence suggests that these microbial partners are also essential contributors to the coral response to both stress and disease. Yet how microbes enhance or weaken coral resistance to stressors, and the degree of specificity of positive associations remains unknown. The recent outbreak of a devastating coral disease (SCTLD) in the Caribbean, which has affected nearly 20 species of corals, has provided unprecedented opportunities to investigate the contributions of holobiont interactions for both disease resistance and response. Here we detail a long-term sampling approach, combining ecological monitoring of individual coral colonies with repeated multi-omic sampling of hosts to identify signatures of both resistance and general host response to disease. A multi-species sampling approach allowed for the investigation of species-specific and species-independent markers of disease resistance and response. Integrating transcriptomic data from hosts and photosymbionts with microbial and viral community composition we identify key holobiont interactions for disease resistance. Furthermore, we highlight species-specific and species-independent shifts in holobiont associations which characterize disease progression. Combined, this powerful dataset significantly advances our understanding coral disease in a holobiont framework.

## **Medical Microbiology Session 1: “Microbial adaptation to host environment”**

## Deciphering the Role of Phospholipases in Lipid Remodeling and Virulence in *Leishmania*

Salma Waheed Sheikh<sup>1</sup>, Somrita Basu<sup>1</sup>, Neha Biyani<sup>1</sup>, Fong-Fu Hsu<sup>2</sup>, Kai Zhang<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Texas Tech University, Lubbock, United States. <sup>2</sup>Department of Internal Medicine, Washington University School of Medicine, Saint Louis, Missouri, United States

### Abstract

*Leishmania* parasites undergo extensive membrane lipid modifications as they transition from the promastigote stage in sandflies to the amastigote stage in mammalian cells. Both promastigotes and amastigotes can acquire lipids by de novo synthesis and scavenging but the identity of lipid remodeling machinery remains unknown. Phospholipases are key enzymes that catalyze hydrolysis of membrane lipids and contribute to nutrient acquisition, signaling and lipid remodeling. While *Leishmania* parasites possess orthologs of phospholipase C (PLC1, PLC2, PLC3) and Lands cycle enzymes (PLA2-B and LPCAT), their roles in phospholipid turnover are understudied. In *Leishmania major*, targeted gene deletions followed by PCR and whole-genome sequencing suggest that PLC1, PLC3, and PLA2-B are essential whereas PLC2 is dispensable. Localization studies revealed diverse subcellular distributions, with PLC1 enriched at the flagellar pocket, the primary site of endo- and exocytosis, PLC2 localized throughout the cytoplasm, and PLA2-B in the endoplasmic reticulum. Lipidomic analysis showed that PLC1 overexpression reduces PE abundance during the stationary phase, implicating the role of PLC1 in phospholipid homeostasis and membrane remodeling. Ongoing virulence studies and DiCre-based knockout attempts will reveal stage-specific functions of these enzymes. Together, these findings will define the critical roles of phospholipid remodeling in *Leishmania* survival and virulence.

## Identification and characterization of a novel *Candida albicans* hyphal regulator, ROH1

Dakota Archambault<sup>1,2</sup>, Giuseppe Buda De Cesare<sup>2</sup>, Luis Vega<sup>2</sup>, Michael Lorenz<sup>1,2</sup>

<sup>1</sup>University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, Houston, United States. <sup>2</sup>University of Texas Health Science Center, McGovern Medical School, Department of Microbiology and Molecular Genetics, Houston, United States

### Abstract

*Candida albicans* is a normal resident of the human microbiota, but defects in the innate immune response can allow this species to disseminate throughout the body, leading to multisystem organ failure and a patient mortality rate of ~40%. How *C. albicans* interacts with phagocytes is therefore a relevant determinant for the development of infection. Its interaction with host macrophages is complex, with hyphal growth as a key feature that contributes to macrophage damage and escape from phagocytosis. Using transcriptomic data from *C. albicans* prior to and after phagocytosis, we identified uncharacterized genes as candidate virulence factors. This set of genes is strongly induced in phagocytosed *C. albicans* cells, and 48% of these have no known function. We generated a mutant library consisting of strains with a deletion in each of these genes; we successfully generated strains for 86 of the 94 genes. Through our screening approach, we identified one hypofilamentous mutant strain in the gene *ROH1* (C2\_04280W), which has hyphal defect phenotypes on solid agar, invasion into an agar surface, and adherence to a solid surface. When deleted, *Roh1*, a putative kinase, exhibits virulence defects in *ex vivo* and *in vivo* models, including macrophage cell lines, *C. elegans* infections, and systemic mouse infections. To further characterize this gene, RNA sequencing was utilized to identify the target of *Roh1*. Altogether, we have characterized a novel regulator in *C. albicans*, which, when deleted, impacts hyphal morphogenesis and virulence of *C. albicans*.

## Pathogen-Induced Metabolic Rewiring Promotes Host Defense During Liquid-Based Bacterial Infection in *Caenorhabditis elegans*.

Maria Fernanda Vaca-Riofrio, Lois Armendariz, Elissa Tjahjono, Yvette Acevedo, Anna Singh, Jane Lowder, Natasha Kirienko

Rice University, Houston, United States

### Abstract

Host defense requires coordinated immune and metabolic responses, yet how pathogen-induced metabolic changes contribute to survival remains poorly understood. Using *Caenorhabditis elegans*, we investigated the role of lipid metabolism during liquid-based pathogenesis by *Pseudomonas aeruginosa* and compared its contribution to defense against the Gram-positive pathogens *Enterococcus faecalis* and *Staphylococcus aureus*.

Transcriptomic analysis revealed that liquid-based *P. aeruginosa* pathogenesis induces a distinct metabolic response characterized by enrichment of lipid metabolism and  $\beta$ -oxidation genes. This transcriptional profile strongly resembled the response to iron deprivation, consistent with a major role for pyoverdine-mediated iron sequestration in driving host metabolic remodeling. An RNAi screen identified 17 lipid metabolism genes required for host survival during liquid-based *P. aeruginosa* pathogenesis. Disruption of these genes also increased susceptibility to *E. faecalis* and *S. aureus*, with susceptibility patterns correlating strongly between the two Gram-positive pathogens.

Mechanistically, liquid-based *P. aeruginosa* exposure triggered cytoplasmic retention of the Max-like factor MXL-3, nuclear localization of the lysosomal transcription factor HLH-30, and downstream lipid droplet depletion. Loss of HLH-30 increased host susceptibility and reduced expression of  $\beta$ -oxidation genes. Together, these findings identify pathogen-induced lipid mobilization as a vital host defense mechanism and establish an MXL-3–HLH-30 regulatory axis linking infection-associated metabolic stress to protective metabolic rewiring.

## Evaluating the impact of human milk oligosaccharides on the vaginal microenvironment

Holly Branthoover, Camille Serchejian, Clare Robertson, Gwendolynn Hummel, Alejandra Rivera Tostado, Sarah Blutt, Kathleen Pennington, Kathryn Patras

Baylor College of Medicine, Houston, United States

### Abstract

The vaginal microbiota is associated with women's health, especially during pregnancy. It serves as a source for neonatal transmission of health-associated *Bifidobacterium* species and pathogens. Current antibiotic prophylaxis limit pathogen transfer but come with off-target microbial alterations and there are few options to promote transfer of beneficial organisms. Human milk oligosaccharides (HMOs), an abundant component in human breast milk, exhibit antibacterial properties against pathogens and prebiotic effects for *Bifidobacterium*. We seek to identify activity of HMOs within the vaginal tract, focusing on interactions with vaginal bacteria and the vaginal epithelium.

We screened vaginal bacteria for the ability to use HMOs in monocultures and co-cultures to assess cross-feeding. We then used human vaginal epithelial organoids (VEOs) to identify how HMOs impact host-microbe interactions by pretreating VEOs with HMOs then exposing to bacteria to interrogate bacterial adhesion and epithelial cytokine response.

Vaginal *Bifidobacterium* displayed increased growth in the presence of HMOs, suggesting their ability to metabolize HMOs, with some isolates releasing glucose. Co-cultures using *B. bifidum*, an isolate that consistently released glucose, showed cross-feeding with both commensal and pathogenic vaginal bacteria. In VEOs, HMO pretreatment reduced adhesion of pathogenic but not commensal microbes and reduced inflammatory IL-8 induction triggered by pathogens.

These results suggest HMOs promote growth of vaginal bacteria that are beneficial to infants while limiting pathogen colonization and inflammation. Ongoing experiments seek to identify cross-feeding driven by HMO utilization of distinct *Bifidobacterium* species. Together, this supports further exploration of HMOs as a therapeutic alternative to promote a health-associated vaginal microenvironment.

## May the force be with you: How EHEC exploits gut mechanics to colonize the GI tract

Anne-Marie Krachler

Department of Microbiology and Molecular Genetics, McGovern Medical School, The University of Texas Health Science Center at Houston, United States

### Abstract

The gastrointestinal tract is a dynamic mechanical environment in which microbes experience fluid flow, shear forces, and peristaltic movements that influence their physiology and behavior. Yet how mechanical cues shape bacterial colonization, and how microbes in turn alter the mechanics of their host niche, remain poorly understood. We use enterohemorrhagic *Escherichia coli* (EHEC) and larval zebrafish to investigate this bidirectional relationship between microbial physiology and host mechanics.

Our studies reveal that EHEC uses mechanical cues to regulate virulence gene expression. In particular, the key regulator GrlA, which controls expression of the type III secretion system, responds to mechanical stimuli by transitioning between membrane-associated and cytoplasmic states, coupling bacterial attachment and mechanical force to activation of the colonization program. To determine how these signals operate in the living host, we combine bacterial transcriptional reporters, live imaging, measurements of intestinal fluid flow, and quantitative analysis of gut motility.

These approaches have also revealed an unexpected consequence of EHEC infection: mechanical cues not only regulate bacterial virulence, but EHEC can actively reshape the mechanical environment of the gut. We find that Shiga toxin 2 promotes changes in intestinal motility that accelerate gut transit. These changes displace resident microbiota and are associated with enhanced EHEC colonization. Together, our findings suggest that EHEC does not simply respond to the physical environment of the intestine, but actively remodels it to create a more favorable ecological niche.

## **Medical Microbiology Session 2: “Pathogenesis and Host Defense”**

## **Csnuc promotes antifolate resistance by facilitating extracellular nucleotide acquisition in *Listeria monocytogenes***

Damilola Oyeboade<sup>1</sup>, Qing Tang<sup>1</sup>, Shoukai Kang<sup>2</sup>

<sup>1</sup>University of Texas at Arlington, Arlington, United States. <sup>2</sup>University of Washington, Seattle, United States

### **Abstract**

*Listeria monocytogenes* (*Lm*) is a foodborne pathogen that causes refractory listeriosis, including meningitis and neonatal stillbirth. Antifolate antibiotics are used to treat infections caused by drug-resistant *Lm* and as an alternative therapy for patients with  $\beta$ -lactam allergies. Their bactericidal activity is attributed to inhibition of thymidylate synthase (ThyA), forcing cells to rely on exogenous thymidine to maintain the dTMP pool, thereby leading to cell death caused by thymidine depletion. Although thymidine is limited within the host cells, *Lm* can evade the bactericidal activity of antifolates by salvaging exogenous nucleotides, which must be dephosphorylated before bacteria utilization. However, the underlying mechanism remains poorly understood. Here, we identified a potential cell-surface nucleotidase/nuclease, which we named CsnuC. We found that CsnuC exhibited both nucleotidases and nucleases activities, enabling it to dephosphorylate nucleotides and DNA cleavage. To determine whether CsnuC facilitates antifolate resistance by salvaging nucleotides, we generated a  $\Delta thyA$  strain as a model of antifolate-mediated ThyA inhibition. Deletion of *csnuc* impaired the survival of  $\Delta thyA$  when dTMP was used as the sole extracellular nucleotide source. Importantly, during oral mouse infection,  $\Delta thyA \Delta csnuc$  exhibited dramatically reduced bacterial burdens compared to  $\Delta thyA$  across intestinal and systemic tissues, suggesting that *csnuc* contributes to  $\Delta thyA$  fitness during infection. Ongoing studies are investigating how *csnuc* influences intracellular spread and survival during antifolate stress. Our findings identify *Csnuc* as a cell-surface protein that enables *Lm* exploit host-derived nucleotides when *de novo* nucleotide synthesis is disrupted by antifolates, providing insights into novel therapeutic targets for combating drug-resistant *Lm* infections.

## Determining the Contribution of a Potential *Streptococcus agalactiae* Virulence Factor During Interaction with Brain Endothelial Cells

Bryan Estrada<sup>1</sup>, Benjamin Klemp<sup>1</sup>, William Cutts<sup>1</sup>, Brandon Kim<sup>1,2,3</sup>

<sup>1</sup>University of Texas at Dallas, Richardson, United States. <sup>2</sup>University of Alabama, Tuscaloosa, United States. <sup>3</sup>University of Alabama at Birmingham, Birmingham, United States

### Abstract

*Streptococcus agalactiae* (GBS) is a Gram-positive pathobiont 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 (BECs) and disrupts the blood-brain barrier. The discovery of GBS factors that contribute to bacterial meningitis has often relied on transposon mutant screens that are complicated by insertion site bias. We 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 non-interacting GBS, enabling the discovery of novel virulence factors. One upregulated transcript, Putative Virulence Factor (PVF), encodes a poorly characterized protein containing a surface anchoring LPXTG motif, indicating a possible role in interaction with host cells. We hypothesize that as PVF is upregulated during GBS interaction with BECs, that PVF contributes to GBS-BEC interaction. A PVF deletion strain ( $\Delta$ PVF) was generated using CRISPR-Cas12 technology, and adherence and invasion assays were conducted using two *in vitro* BEC models. Compared to WT control,  $\Delta$ PVF exhibited significantly reduced invasion and adherence with hCMEC/D3 cells and adherence to iBECs, suggesting a role for PVF in infection of BECs. Additionally, scanning electron microscopy qualitatively revealed reduced aggregation by  $\Delta$ PVF GBS compared to WT. We recently generated a complemented  $\Delta$ PVF strain to further characterize PVF's contribution to GBS-BEC interaction. Here represents a novel method for discovery of GBS virulence factors and their contribution to disease progression.

## A Neuropeptide F and FoxA axis regulates stem cell mediate parasite development

Sabona Simbassa, Ryan Sloan, Pallavi Yadav, Jun Dae Kim, Jayhun Lee

UTHealth-Houston, HOUSTON, United States

### Abstract

*Schistosoma* is a parasitic flatworm that dwells within the bloodstream and feeds on blood to support parasite development and homeostasis. Previous work identified that the forkhead transcription factor FoxA is enriched in the esophageal gland (EG), a specialized organ essential for blood processing and EG maintenance. Ablation of *foxA* via RNAi in adult parasite results in stunted growth and reduced stem cell proliferation, suggesting that FoxA plays a role in stem-cell driven parasite development, however the mechanism remains elusive. Here, we investigated the tissue-specific roles of *foxA* and its role in parasite development. We discovered that *foxA* is enriched in a subset of neurons and somatic *eled+* neoblasts. The loss of *foxA* in larval worms results in a significant reduction of *eled+* neoblast. In parallel, these worms were significantly shorter, with motility defects and an enlarged gut. Transcriptomic analysis of FoxA-dependent host blood-independent genes uncovered top candidate regulators expressed in the EG, neurons, and *eled+* neoblasts. An RNAi screen of candidate genes enriched in the EG or neurons identified neuropeptide F (*npf*) that phenocopied *foxA* RNAi worms, showing loss of EdU+ cells, loss of *foxA* and EG gene expression, and a feeding defect. A subset of neurons within the brain express *npf*, however, this expression does not co-localize with *foxA+* neurons. This suggests that NPF likely signals via a cognate receptor to regulate FoxA-dependent schistosome development. Our work provides insights into the neural regulation of flatworm development and opportunities to target immature parasites via the NPF signaling pathway.

## Identification of Immunogenic Peptide Targets for Improved Toxocariasis Diagnosis

Jacob J. Williams<sup>1,2</sup>, Nina L. Tang<sup>2,3</sup>, Nestor Uzcategui Araujo<sup>2</sup>, Angela Jacobo<sup>2</sup>, Sukwan Handali<sup>4</sup>, Leroy Versteeg<sup>2</sup>, Jeroen Pollet<sup>2,3</sup>, Rakesh Adhikari<sup>2</sup>, Maria Elena Bottazzi<sup>2,3</sup>, Bin Zhan<sup>2</sup>, Jill Weatherhead<sup>2,5</sup>, Megan Duffey<sup>2,5</sup>

<sup>1</sup>Rice University, Houston, United States. <sup>2</sup>Department of Pediatrics, Division of Pediatric Tropical Medicine and Center for Vaccine Development, National School of Tropical Medicine, Baylor College of Medicine, Houston, United States. <sup>3</sup>Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, United States. <sup>4</sup>US Centers for Disease Control and Prevention, Atlanta, United States. <sup>5</sup>Department of Medicine, Baylor College of Medicine, Houston, United States

### Abstract

Toxocariasis is a parasitic infection caused by *Toxocara* species roundworms and is a significant public health threat, particularly to children in resource-limited settings. Clinical presentations vary widely, and prolonged infection can lead to serious complications, including irreversible vision loss and organ damage. Clear diagnosis remains challenging because the current gold standard assay relies on crude *Toxocara* excretory-secretory (TES) antigens and cannot reliably distinguish active infection from prior exposure. Building upon previous work exploring the recombinant antigens Tc-CTL-1, Tc-CTL-2, and TES-26, we aimed to develop a novel semi-quantitative recombinant immunoassay for toxocariasis diagnosis and clinical monitoring. We evaluated these full-length recombinant proteins using ELISA and western blot with sera from *Toxocara*-seropositive and -seronegative individuals and found that all three proteins demonstrated limited specificity. To identify more specific immunogenic targets, we performed linear epitope mapping across the three proteins. This analysis identified 32 partially overlapping 15-mer peptides that were reactive with human sera from *Toxocara*-exposed individuals but not unexposed controls, demonstrating higher specificity than the full-length proteins. These peptides were clustered into 11 distinct immunogenic regions. Furthermore, AlphaFold3 and ChimeraX structural protein modeling showed that each of these regions were surface-exposed in their native proteins, supporting their immunogenicity. These findings thus identified candidate peptide targets for the development of more specific semi-quantitative immunoassays. Future studies will evaluate these peptides in ELISA and Luminex platforms, followed by analysis of longitudinally collected human sera before and after toxocariasis treatment to determine whether these assays can distinguish active infection from prior exposure.

## The chemical basis of host-helminth interactions

Navonil Banerjee

Department of Microbiology and Molecular Genetics, McGovern Medical School, The University of Texas Health Science Center at Houston

### Abstract

Parasitic helminths infect approximately 2 billion people worldwide, yet the mechanisms driving their infectivity remain obscure. These parasites encounter multiple host-derived chemicals throughout their life cycle. Do these chemicals act as sensory or metabolic cues to support their life cycle and promote infections? We are studying the role of carbon dioxide (CO<sub>2</sub>), a host-derived cue, in promoting host-parasite interactions using the human-parasitic gastrointestinal helminth *Strongyloides stercoralis*. Specifically, we are investigating the role of CO<sub>2</sub> in (a) host seeking, (b) intra-host development, and (c) intra-host migration to reach the site of infection.

Our results show that *S. stercoralis* display flexible sensory preferences to CO<sub>2</sub> across distinct life stages. Depending on the specific life stage, this may enable these parasites to initiate host seeking or migrate to and remain within high-CO<sub>2</sub> tissues (such as the small intestine) inside the host to establish infections. Using genetic tools, we have also identified a pair of sensory neurons and a molecular sensor that enable these parasites to detect and respond to CO<sub>2</sub>. Our study provides mechanistic evidence that parasitic helminths detect and respond to host-derived chemosensory cues. Future studies aim to determine how CO<sub>2</sub> influences parasitism and to identify additional host-derived chemical cues that support helminth life cycles and promote infections. Our work will provide fundamental insights into the chemical basis of host-helminth interactions and will inform the design of new anthelmintic drugs that disrupt crucial interactions of helminths with host-derived chemical cues.

## **Scientific Session (Plenary): “Decoding Microbiology Systems: Antibiotics, Phages and AI”**

## Decoding Bacterial Pathogenesis with Big Data and AI

Jing Zhang

Department of Pediatrics, University of Texas Southwestern Medical Center

### Abstract

Bacterial pathogens employ diverse virulence factors and molecular mechanisms to colonize hosts, adapt to changing environments, evade host defenses, and cause disease. The rapid expansion of bacterial genomic data offers new opportunities to discover genes underlying pathogenicity, but translating this wealth of information into a systematic understanding of the molecular mechanisms of bacterial pathogenesis remains a major challenge. My previous research has addressed this challenge through complementary approaches spanning comparative genomics, experimental phenotyping, AI-assisted protein-protein interaction prediction, and structural modeling.

To investigate pathogenic mechanisms in inflammatory bowel disease-associated *Escherichia coli*, we integrated pangenome analysis with experimental phenotyping to identify accessory genes and metabolic pathways associated with epithelial invasion, linking strain-specific genomic variation to pathogenic phenotypes. Meanwhile, inspired by recent advances in AI, we developed RoseTTAFold2-Lite, a rapid deep learning model that leverages coevolutionary signals between protein pairs to enable proteome-wide protein-protein interaction (PPI) screening. Using this pipeline, we screened 78 million protein pairs across 19 human bacterial pathogens and predicted numerous previously unrecognized PPIs involving essential proteins and virulence factors, with top candidate interactions experimentally validated. We further extended this interaction-based strategy from proteome-wide discovery to the targeted investigation of specific virulence systems. Specifically, we used structurally conserved but sequence-divergent type III secretion system chaperones as baits to identify candidate effectors and regulators that interact with these chaperones.

Building on this foundation, my laboratory aims to integrate *in silico* analyses with experimental validation to transform computational predictions into mechanistic biological insights.

## Metabolic impacts of bacteriophages on anaerobic phototrophic bacteria in early Earth analogs

Jordan Walker<sup>1</sup>, Natascha Varona<sup>2</sup>, Bailey Wallace<sup>3</sup>, Alice Bosco-Santos<sup>4</sup>, Molly O'Beirne<sup>5</sup>, Josef Werne<sup>5</sup>, William Gilhooly<sup>6</sup>, Cynthia Silveira<sup>3</sup>

<sup>1</sup>Sam Houston State University, Huntsville, United States. <sup>2</sup>UC Berkley, Berkley, United States.

<sup>3</sup>University of Miami, Miami, United States. <sup>4</sup>UNIL, Lausanne, Switzerland. <sup>5</sup>University of Pittsburgh, Pittsburgh, United States. <sup>6</sup>Indiana University, Indianapolis, United States

### Abstract

The structure of microbial communities and the stable conditions in meromictic lakes make them powerful analogs for ancient oceans and astrobiology. The dominant primary producers in these systems are purple sulfur bacteria (PSB) and/or green sulfur bacteria (GSB), which leave characteristic carbon and sulfur isotopic signatures and diagenetic pigment products in the geologic record. Observations from meromictic lakes reveal a decoupling between biomarkers of sulfide-oxidizing bacteria and bulk carbon isotope signatures, suggesting additional ecological controls. Viruses infecting bacteria can alter host metabolism and may contribute to the observed decoupling. Here, we utilize meta'omics techniques to characterize virus-host interactions and their metabolic impacts at three meromictic lakes in the North American Pacific Northwest. In PSB-dominated lakes, PSBs account for 73% of community gene expression, significantly higher than their relative abundance of 30%. In a GSB-dominated lake, GSBs represent 30% of the community and only 20% of the activity. We show that PSB lakes have significantly higher abundance of temperate viruses, those that establish dormant, long-term infections, and bacteria containing viral defense systems. Furthermore, all viruses infecting PSB were classified as temperate, while those linked to GSB were lytic, i.e., causing cell death upon infection. Viruses in both lake types encoded genes implicated in sulfate reduction to sulfite and pathways for heme incorporation into pigments. Our results provide evidence that differences in viral lifestyles influence the decoupling of host abundance and activity and demonstrate viruses' potential to directly alter host metabolic pathways involved in pigment production and isotopic biosignature formation.

## Nasal colonization by *Klebsiella pneumoniae* promotes gastrointestinal carriage

Rina Matsuda<sup>1</sup>, A. Scott Bray<sup>1</sup>, Nathanael Mathew<sup>1</sup>, Alfredo Calderon-Macedo<sup>1</sup>, Noah Bassinger<sup>1</sup>, Mohamed Kady<sup>1</sup>, George Balazs<sup>2</sup>, Matthew Waldor<sup>2</sup>, Kathryn Patras<sup>1</sup>, Karthik Hullahalli<sup>3</sup>, Robert Britton<sup>1</sup>, Caitlyn Holmes<sup>4</sup>

<sup>1</sup>Baylor College of Medicine, Houston, United States. <sup>2</sup>Harvard Medical School, Boston, United States.

<sup>3</sup>Loyola University Chicago Stritch School of Medicine, Chicago, United States. <sup>4</sup>, University of Minnesota Medical School, Minneapolis, United States

### Abstract

*Klebsiella pneumoniae* (Kp) is a leading cause of potentially life-threatening septicemia and pneumonia, posing a major concern for global health due to extensive multi-drug resistance. A major risk factor for Kp infection is carriage in the gastrointestinal tract, as the strains colonizing the gut and causing infection match in about 80% of cases. Despite the importance of the gastrointestinal tract as a reservoir for Kp infection, mechanisms that facilitate establishment of Kp gut colonization are incompletely understood. Interestingly, Kp also colonizes the nasal cavity in 1-20% of humans. Unlike intestinal colonization however, the contribution of nasal carriage to infection of secondary sites is uncharacterized.

Here, we show that Kp nasal colonization supports gastrointestinal carriage in a murine model. Oral pipette feeding of Kp established persistent colonization of the nasal cavity and gastrointestinal tract for multiple weeks. Strikingly, tracking barcoded bacteria using Sequence Tagged Analysis of Microbial Populations with Resampling (STAMPR) revealed that nasal and intestinal populations were highly similar with few founding clones. Bypassing nasal inoculation by small-volume feeding of an equivalent bacterial dose reduced gut carriage, and large-volume intranasal inoculation was sufficient for sustained nasal and intestinal colonization. Finally, bacterial capsule was required for colonization of both sites. Together, these observations reveal that the nasal passage is a previously unappreciated niche for Kp that promotes gastrointestinal pathogen carriage. Findings will inform new decolonization strategies to remove pathogens carried asymptomatically prior to tissue invasion, mitigating serious infections with antibiotic resistant organisms.

## **A Capsular Polysaccharide Transporter Couples Capsule Assembly with Gliding Motility**

Ran Wang<sup>1</sup>, Francesco Renzi<sup>2</sup>, Yongtao Zhu<sup>3</sup>, Bo Hu<sup>1</sup>

<sup>1</sup>Department of Microbiology and Molecular Genetics, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth Houston), Houston, United States.

<sup>2</sup>Research Unit in Biology of Microorganisms (URBM), Namur Research Institute for Life Sciences (NARILIS), University of Namur, Namur, Belgium. <sup>3</sup>Department of Biosciences and Bioinformatics, School of Science, Xi'an Jiaotong-Liverpool University, Suzhou, China

### **Abstract**

*Flavobacterium johnsoniae*, a member of the Bacteroidetes, moves over surfaces by gliding motility, driven by adhesins propelled along a gliding track. Many bacteria export capsular polysaccharides (CPS) across the outer membrane through outer-membrane polysaccharide export (OPX) proteins. These processes are considered distinct. Here we show that a CPS export translocon interacts directly with the gliding track. Cryo-EM single-particle analysis of a ~2 MDa supercomplex at 3.1 Å reveals an OPX protein, Fjoh\_0353, bound to the GldKJN gliding track. Twelve Fjoh\_0353 protomers form a closed channel with a spiral-staircase arrangement. GldN interacts with the basal face of its periplasmic domain, the surface used by other OPX translocons to bind their co-polymerase. Fjoh\_0353 exports CPS to the cell surface. Cells lacking it have no surface CPS, adhere more strongly to glass, and are impaired in gliding. CPS export proceeds without an intact track, whereas gliding is impaired without CPS. These findings show that machineries for polysaccharide secretion and gliding motility are built into a shared assembly in the bacterial cell envelope.

## The Iron Age of Antibiotics

William Miller

Division of Infectious Diseases at Houston Methodist Hospital, Houston Methodist Academic Institute, Weill Cornell Medical College

### **Abstract**

*Pseudomonas aeruginosa* is an opportunistic human pathogen and a frequent cause of multidrug-resistant infections. The novel siderophore-cephalosporin conjugate antibiotic Cefiderocol was designed to overcome both the intrinsic and acquired  $\beta$ -lactam resistance mechanisms of *P. aeruginosa*. Cefiderocol retains high rates of *in vitro* activity against carbapenem-resistant *P. aeruginosa* and is being introduced as a last resort antibiotic in developing countries; however, the emergence of resistance is a growing problem. This talk will review how iron homeostasis in *P. aeruginosa* influences the uptake of cefiderocol, the mechanistic bases of cefiderocol resistance prevalent in clinical isolates, and therapeutic strategies to mitigate the emergence of resistance and preserve the utility of this important antibiotic.

## Poster Abstracts

## **General Microbiology Undergraduate Student Posters**

## ***Escherichia coli* formaldehyde response requires fatty acid degradation to recycle damaged lipids**

Erum Ahmed<sup>1</sup>, Priya Christensen<sup>1</sup>, Richard Ratevosyan<sup>1</sup>, Phillipe Zimmern<sup>2</sup>, Kelli Palmer<sup>1</sup>, Larry Reitzer<sup>1</sup>, Jacob Hogins<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, The University of Texas at Dallas, Richardson, United States.

<sup>2</sup>Department of Urology, University of Texas Southwestern Medical Center, Dallas, United States

### **Abstract**

*Escherichia coli* causes ~75% of urinary tract infections (UTIs), of which 25% recur in part due to antibiotic resistance. As such, non-antibiotic treatments are needed to minimize recurrence. One such non-antibiotic treatment is Hiprex, a medication that releases the toxic antimicrobial agent formaldehyde in the bladder. A previous transcriptomic result in the lab suggested that formaldehyde attacks proteins, nucleic acids, and unexpectedly lipids in pathogenic *E. coli*. We hypothesize that **formaldehyde exposure alters the membrane's lipid composition, and genes involved in fatty acid degradation pathways are essential for the response to formaldehyde in both pathogens and non-pathogenic *E. coli*.**

For three *E. coli* strains cultured with and without formaldehyde, we isolated and quantified lipids, determined the ratio of saturated to unsaturated lipids, and visualized the lipids species using thin layer chromatography. These analyses confirmed our hypothesis: alterations in the phospholipids and fatty acids occur due to formaldehyde exposure. To see if lipid recycling genes are expressed during formaldehyde exposure, we extracted RNA from both control and formaldehyde-treated cultures of a non-pathogenic *E. coli*. The formaldehyde group expressed *fadD* 3.5-fold greater than the untreated samples. Furthermore, a *fadD* mutant took longer to recover from formaldehyde exposure, which is consistent with a role in recovery. Other mutants had expected phenotypes: for example, loss of formaldehyde detoxification genes prevented recovery. Taken together, we have shown that in addition to protein and nucleic acids, formaldehyde attacks lipids, and that recovery from formaldehyde intoxication involves fatty acid degradation to presumably remove the damaged lipids.

## Formulation and Characterization of Synthetic Lipid Droplets Loaded with the Novel Antimicrobial - Silver Ibuprofen.

Junyoung Ahn, Ricardo Muñoz-Medina, Ashlyn Wolf, Carolyn Cannon

Texas A&M University, Naresh K. Vashist College of Medicine, Bryan, United States

### Abstract

*Pseudomonas aeruginosa* (Pa) is a Gram-negative opportunistic pathogen with growing resistance to standard-of-care antimicrobials, and consequently, has been designated as a serious threat by the World Health Organization. Thus, alternative therapeutic formulations to combat these resistant strains are needed. Biocompatible lipid droplets (LDs) are promising drug delivery devices for water-insoluble drugs. Hydrophobic drugs can be loaded into the LD core that provides protection from enzymatic degradation or other harsh conditions. Additionally, LDs can be coated with ligands that target them to tissues or cells including bacteria. Targeting to bacteria results in accumulation at the site of infection, which may overcome antimicrobial resistance. We aimed to develop a LD formulation containing the novel antimicrobial, silver ibuprofen (AgIBU), for the treatment of antimicrobial-resistant Pa infections. Ideally, LDs are composed of a neutral lipid core surrounded by a single phospholipid monolayer. We characterized our LDs by dynamic and electrophoretic light scattering, confocal microscopy, and thin-layer chromatography. The LDs are spherical with a diameter of ~180 nm and a zeta potential of -8.17 mV. The ratio of phospholipids to neutral lipids was 9% confirming a single phospholipid layer. Confocal microscope studies of Nile Red-loaded LDs confirmed the neutral lipid core. Loading AgIBU into LDs (AgIBU-LDs) resulted in 60 µg/mL AgIBU by weight with stable release. The minimum inhibitory concentration of the AgIBU-LDs against resistant Pa isolates was 2 mg/mL. Thus, we have formulated lipid droplets loaded with silver ibuprofen that may effectively treat infections caused by resistant *Pseudomonas aeruginosa*.

## Genomic Characterization of Cluster BI *Streptomyces lividans* Bacteriophage, Monse

Adriana Carrizales, Lee Hughes

University of North Texas, Denton, United States

### Abstract

Bacteriophages (phages) are viruses that infect bacterial hosts and play a critical role in microbial diversity and evolution. In this study, bacteriophage Monse, was isolated from soil samples collected in the North Texas region using *Streptomyces lividans* as the host. The phage was isolated, purified, and amplified following standard SEA-PHAGES protocols. Plaque morphology revealed a distinct phenotype among Monse, with the formation of clear, circular plaques averaging 1 mm. While Monse belongs to cluster BI, it also belongs to sub-cluster BI2, indicating the genomic diversity of the BI cluster group.

Genome sequencing was performed using Illumina NextSeq 1000 with single-end 100 bp reads, achieving a coverage range of 189x for this phage. Monse had a considerably sized genome (43,522 bp), highlighting notable size variation within the BI cluster. The phage presented with a 3' sticky overhang of 9 base pairs, while also displaying a slight variation (CGCCGCCCT). GC content for Monse resulted at 60.4%, consistent with cluster BI characteristics.

Genome manual annotation identified 58 predicted genes in Monse, respectively, with no tRNAs detected. A large proportion of predicted genes currently lack functional annotation, highlighting the genetic novelty of this phage. This work expands the catalog of BI cluster phages and provides insight into genomic diversity within closely related *Streptomyces* bacteriophages, contributing to a broader understanding of phage evolution and host interactions.

## Lumify-Induced Changes in Bacterial Competition and Their Potential Implications for the Ocular Microbiome

Kaitlyn Foerster, Samma Ali, Jorge Guzman, Adi Pathak

Texas Tech University, Lubbock, United States

### Abstract

Lumify eye drops are a commonly used cosmetic vasoconstrictor intended to reduce redness, making the eyes appear whiter and brighter. Although Lumify is recommended for occasional use, some individuals use it daily and report symptoms including increased redness, burning, blurred vision, and dryness. We hypothesized that the ingredients in Lumify eye drops may be disrupting the ocular microbiome, which is often associated with these symptoms. Lumify contains brimonidine tartrate and benzalkonium chloride, both of which possess antimicrobial properties. Because of these properties, repeated exposure may influence bacterial growth and competition within the ocular microbiome. This study investigated how Lumify affects the competition between the gram-positive and gram-negative bacteria commonly associated with the ocular surface using model bacterial species that are representative of or identical to species found on the eye. A bracket-style elimination design was employed wherein bacterial cultures were grown in the presence and absence of Lumify to evaluate its effects on bacterial growth, and species that demonstrated greater growth moved onto the next round for comparison with other competitors. We found that Lumify inhibited gram-positive bacterial growth and selectively suppressed the growth of certain gram-negative bacterial species including *P. stutzeri* and *E. aerogenes*. These findings suggest that repeated Lumify exposure may disrupt the microbial balance of the ocular surface. This warrants further investigation into its overall effects on the ocular microbiome.

## Investigating Role of *epaI* Gene Mutations in Resistance of Hormonemate in *Enterococcus faecalis*

Alannah Grimes<sup>1,2</sup>, Domenica Acevedo-Lopez<sup>1,2</sup>, Christian Hobson<sup>3</sup>, Sarah W. Johnson<sup>1,2</sup>, Wenliang Wang<sup>3</sup>, Truc T. Tran<sup>1,2,4</sup>, Rodrigo de Paula Baptista<sup>1,2,4</sup>, Guillermo Hoppe-Elsholz<sup>1,2</sup>, Diana Panesso<sup>1,5,4</sup>, Kavindra V. Singh<sup>1,2,4</sup>, Gerry Wright<sup>3</sup>, Cesar A. Arias<sup>1,2,4</sup>

<sup>1</sup>Center for Infectious Diseases, Houston Methodist Research Institute, Houston, Texas, United States. <sup>2</sup>Division of Infectious Diseases, Houston Methodist Hospital, Houston, Texas, United States. <sup>3</sup>Michael G. DeGroote Institute for Infectious Diseases Research, McMaster University, Hamilton, Ontario, Canada. <sup>4</sup>Department of Medicine, Weill Cornell Medical College, New York, New York, United States. <sup>5</sup>Division of Infectious Diseases, Houston Methodist Hospital, Houston, United States

### Abstract

**Background:** Enterococci are clinically challenging pathogens due to resistance to many available antimicrobials. In *E. faecalis*, daptomycin (DAP) resistance is mediated by the LiaFSR cell envelope stress response system, and disruption of LiaFSR restores DAP susceptibility in enterococci. Our previous work identified HMX as a compound that synergizes with DAP. Selection for HMX resistance resulted in DAP-hypersusceptibility through LiaFSR inactivation. During HMX adaptation, two *epaI* variants, *epaI*<sup>622C>T</sup> and *epaI*<sup>Δ460-486</sup>, emerged in HMX-resistant, DAP-hypersusceptible *E. faecalis*. However, the contribution of *epaI* to HMX resistance remains unclear.

**Methods:** To assess the impact of *epaI* variation on HMX and DAP susceptibility, allelic exchange was performed in *E. faecalis*. The *epaI*<sup>622C>T</sup> and *epaI*<sup>Δ460-486</sup> alleles were exchanged between HMX-resistant isolates selected under different conditions. Additionally, each variant was introduced into an HMX-susceptible, DAP-susceptible background to replace *epaI*<sup>WT</sup>. Mutagenesis was performed using the PheS\* counterselection system and pHOU1. HMX and DAP MICs were determined by broth microdilution and gradient diffusion methods, respectively.

**Results:** Allelic replacement successfully exchanged the two *epaI* variants between HMX-resistant strains. HMX resistance and DAP hypersusceptibility were retained following exchange of *epaI*<sup>622C>T</sup> and *epaI*<sup>Δ460-486</sup>. In contrast, repeated attempts to replace *epaI*<sup>WT</sup> with either mutant allele in an HMX-susceptible, DAP-susceptible background were unsuccessful.

**Conclusion:** These findings suggest that *epaI*<sup>622C>T</sup> and *epaI*<sup>Δ460-486</sup> contribute similarly to the HMX-resistant phenotype. Furthermore, the inability to introduce either mutant allele into a strain with an intact LiaFSR system suggests that *epaI*-mediated adaptation may require additional genetic changes associated with HMX resistance or LiaFSR inactivation.

## Unraveling Weaknesses in Efflux-driven Antibiotic Resistance in *E. coli* using CRISPRi

Jeremy Hernandez<sup>1,2</sup>, Abhishek Anand<sup>2</sup>, Souvik Bhattacharyya<sup>2</sup>

<sup>1</sup>The University of Texas at San Antonio, San Antonio, United States. <sup>2</sup>The University of Texas Health Science Center at Houston, Houston, United States

### Abstract

Antimicrobial Resistance (AMR) is a global health concern, with Gram-negative pathogens causing the greatest death toll, projected to increase further. Multidrug efflux pumps are a dominant mechanism of AMR, exporting toxic compounds and reducing lethal intracellular antibiotic concentrations. Because efflux pumps are nonessential and functionally redundant, targeting them therapeutically is challenging. Here, we investigated whether loss or gain of efflux activity reveals genes conditionally essential for *E. coli* fitness. We hypothesized that altered efflux activity increases cellular stress by affecting envelope stability, energy metabolism, and protein folding. Efflux gene deletions from the Keio collection were transduced into the CRISPRi-dCas9 WT strain (LC-E75) and amplicon sequence-verified. We chose WT,  $\Delta tolC$ , and  $\Delta marR$  to represent baseline, low, and high efflux capacity, respectively, as confirmed by minimum inhibitory concentration assays. A genome-wide CRISPRi library was transformed into each strain, and guide abundance was compared between initial and final time points under four conditions: no antibiotic, sublethal ciprofloxacin, chloramphenicol, and novobiocin. We identified distinct conditional-dependency candidates across efflux backgrounds and antibiotic conditions. In the high-efflux background, *cmoB*, *nagB*, *slyB*, and *lpp* genes involved in tRNA modification, envelope integrity, metabolism, and lipoprotein biosynthesis, respectively, became conditionally essential. In the low-efflux background, *yedK*, *adhE*, and *aidC*, genes important for genome protection and redox balance, were conditionally essential. These findings provide a proof of concept that altered efflux activity creates conditional genetic dependencies across multiple cellular pathways, supporting the hypothesis that efflux state broadly reshapes cellular stress responses.

## Transcriptomic analysis of thermal adaptation in *P. aeruginosa* biofilms

Angelina Marranzini, Alex Luecke, Catherine Wakeman

Texas Tech University, Lubbock, United States

### Abstract

The bacterium *Pseudomonas aeruginosa* is a Gram-negative pathogen that forms biofilms in infections of burn victims, open wounds, and the lungs of cystic fibrosis patients. The biofilm protects the bacteria from the immune system and antibiotics, making these infections extremely difficult to treat and potentially deadly for patients. *P. aeruginosa* can thrive in various niches of different temperatures. Therefore, we are interested in understanding how it adapts to thermal stress. In previous research, we analyzed gene expression in PA14 wild-type biofilms grown at 23 °, 30 °, 37 °, and 40 °C and processed them for RNA-seq analysis. Our findings indicate that PA14 biofilm expression profiles are distinct in response to temperature. Here we highlight some select genes, *intT*, *vapI*, *coxA*, *Fha2*, and *PA14\_21120*, that were differentially expressed in our study. In future studies, we will compare the expression profile of these genes in other *P. aeruginosa* strains, such as PAO1 and PAK. We hypothesize that the gene expression of the biofilms of PAK and PAO1 grown at 23 °C and 37 °C will be similar to the gene expression of PA14 wildtype. To study this, we are harvesting the biofilms of strains of PA14, PAK, and PAO1 in both 23 °C and 37 °C for 48 hours for RNA extraction and subsequent RT-PCR and qPCR analysis. The results of this experiment could help identify conserved versus strain-specific temperature-dependent pathways of *Pseudomonas aeruginosa* at 23 °C and 37 °C to improve current treatments of the infections.

## **CRISPR/Cas12a-Mediated Chromosomal Integration of Fluorescent Reporters in *Streptococcus agalactiae***

Ashley McDonald, Aryah Lee, William Cutts, Brandon Kim  
University of Texas at Dallas, Richardson, United States

### **Abstract**

*Streptococcus agalactiae* (Group B Streptococcus, GBS) is a Gram-positive opportunistic pathogen that remains a leading cause of severe neonatal infections worldwide. With no approved vaccine, improved genetic tools will advance GBS pathogenesis studies and support preventative strategies. Genetic manipulation of GBS remains challenging, requiring alternative genome-editing approaches. We hypothesized that chromosomal insertion of fluorescent proteins will enable visualization of GBS in live cell culture with stable expression. Using CRISPR/Cas12a, we investigated chromosomal integration of genes encoding fluorescent proteins, including green fluorescent protein (GFP), mScarlet, mKate, and mTurq to reduce limitations associated with plasmid loss during bacterial replication. A plasmid-based Cas12a genome-editing strategy was designed containing a guide RNA (sgRNA) that guides Cas12a to the genomic insertion site and a repair cassette containing the fluorescent gene of interest flanked by homology arms. Upon transformation into GBS, Cas12a expression was induced using anhydrotetracycline (aTc), allowing the sgRNA to guide Cas12a to the chromosomal integration site and generate a double stranded break. Surviving colonies were screened by PCR and gel electrophoresis for chromosomal insertion of the fluorescent reporter gene. Chromosomal integration of mScarlet knock-in was confirmed through molecular screening, demonstrating the effectiveness of the CRISPR/Cas12a system for introducing fluorescent reporters into GBS. This system can be further applied to generate additional fluorescent knock-ins, including mKate and mTurq. These findings support CRISPR/Cas12a for introducing stable fluorescent reporters into GBS. Expanding this approach may improve genetic tools for visualizing GBS, investigating genes and proteins during infection, and furthering research on GBS pathogenesis.

## Biochemical and Genetic Analysis of the Regulators of Alanine Biosynthesis in *Escherichia coli*

Mithun Mekala, Jacob Hogins, Lawrence Reitzer

University of Texas at Dallas, Richardson, TX, United States

### Abstract

Because alanine is required for both protein and peptidoglycan synthesis, the regulation of alanine transaminase expression in *Escherichia coli* provides insight into how bacteria coordinate these processes with metabolism. Glutamate-dependent transaminases synthesize alanine from pyruvate, and *E. coli* has two genes that code for alanine transaminases, *alaA* and *alaC*.

We investigated the binding of known pyruvate- and alanine-binding transcription factors, PyrR and PdhR, and a regulator of cell wall synthesis, MraZ, to the promoter regions of *alaA* and *alaC*. We successfully isolated PyrR, PdhR, and MraZ: a single band on gel electrophoresis and western blot of the his-tag extension.

We performed mobility shift assays to detect protein-DNA interactions were performed with and without pyruvate and alanine. MraZ bound to *alaA* DNA, which pyruvate inhibited and alanine stimulated. PyrR and PdhR bound to *alaC* DNA, and neither pyruvate nor alanine affected this binding.

We are currently constructing strains with fusions of the *alaA* and *alaC* promoter regions to GFP to analyze gene expression in strains lacking each of the proposed regulators. These results will allow us to compare genetic and biochemical results, and to examine impacts of different stressors. Further understanding of alanine synthesis could suggest possible targets for antimicrobial development.

## Adaptation to Nutrient-Rich Complex Medium Has a Cost in Alcohol Stress

Julian Ramirez, Brent Orji, Wei-chin Ho

The University of Texas at Tyler, Tyler, United States

### Abstract

Adaptive mutations improve an organism's fitness in one environment, but the effects of the mutations may change when environments shift. 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 previous mutations retain their advantages, organisms may transition more easily into new environments. To explore this question, we examined three distinct bacterial populations that had evolved in Luria-Bertani (LB) medium for 900 days. The populations accumulated mutations that improved their fitness within this specific environment. To determine whether these derived mutations were beneficial, neutral, or harmful in new stressful environments, we transferred the adapted microbes into three new stressful environments: LB broth with 4% ethanol, LB broth with 3% isopropanol, and LB broth with both 2% ethanol and 1.5% isopropanol. The results showed a general trade-off between environments with and without stress; historically beneficial mutations became disadvantageous. The results indicate that microbes thriving in LB broth struggled in new stressful environments.

Interestingly, we found that the antibiotic abilities of ethanol and isopropanol are synergistic. To further understand the underlying molecular basis, we examined the profile of derived mutations in more detail. We will discuss candidate mutations that can explain this cross-environmental trade-off.

## **Environmental Microbiology Undergraduate Student Posters**

## Characterizing the FlhSR regulatory pathway in the soil bacterium *Paracoccus denitrificans*

Umair Bhat, Saim Mughal, Trusha Parekh, Stephen Spiro

The University of Texas at Dallas, Richardson, United States

### Abstract

*Paracoccus denitrificans* is a methylotrophic soil bacterium that can use one-carbon compounds such as methanol and formaldehyde as sources of energy and carbon. Two regulatory proteins, FlhS and FlhR, activate expression of genes encoding enzymes required for metabolism of these compounds. In the absence of FlhS or FlhR, *P. denitrificans* cannot grow on C1 compounds. To further explore this two-component regulatory system, we assessed the possible role of the phosphotransferase (Hpt) protein. We hypothesized that Hpt is required to carry the activation signal from FlhS to FlhR. We also hypothesized that FlhSR is required for expression of two proteins involved in carbon fixation: fructose-1,6-bisphosphatase (Fbp) and the regulatory protein CbbR. Understanding these regulatory systems may help identify toxins and explain bacterial adaptation to extreme conditions. To study Hpt requirement, we deleted the *hpt* gene and assayed growth of *hpt* mutant to compare to wild type on methylotrophic substrates. The *hpt* mutant grew at the same rate on methanol as wild type. Formaldehyde dehydrogenase activity in  $\Delta hpt$  extracts was comparable to wild type, as well. These results suggest that Hpt is not required for methylotrophic growth and is not involved in the FlhSR-mediated signal transduction pathway. Next, we generated *fbp* and *cbbR* promoter-lacZ reporter fusions and introduced them into *P. denitrificans*.  $\beta$ -galactosidase assays will determine whether these promoters are activated during methylotrophic and autotrophic growth. We expect strong activation in wild type on C1 substrates and will determine whether CbbR and FlhSR regulate *fbp* transcription under these conditions.

## Differential Antibiotic Production by Soil Isolates Cultured on Diverse Media

Jeremy Cattoor, Aaron Lynne

Sam Houston State University, Huntsville, United States

### Abstract

Infections caused by antibiotic resistant bacteria are increasingly becoming more prevalent globally, often making common infections life-threatening. In addition, the rate of new antibiotic discovery is declining. To combat this problem, a course-based undergraduate research experience was developed for microbiology labs where bacteria is isolated from the soil and screen for antibiotic production against various bacteria. In this study, 50 soil isolates were screened for antibiotic production against six bacteria (*Escherichia coli*, *Erwinia caratovora*, *Pseudomonas putida*, *Bacillus subtilis*, *Enterococcus raffinose*, and *Enterococcus aerogenes*) on four different media types (LB agar, Difco Plate Count Agar, R2A agar, and BUG agar) for differential antimicrobial production. Results showed that several isolates produced antibiotics on all media types but were not effective against all bacteria screened. Interestingly, several isolates only displayed antibiotic production on 1 or 2 media types. Future research will involve trying to determine the cause of differential antibiotic production.

**Session A - UG P7 (Board 7)**

**Genome Sequencing of *Staphylococcus aureus* Isolated from Phone Cases**

Isabella Howard<sup>1</sup>, Aaron Lynne<sup>2</sup>

<sup>1</sup>Sam Houston State University, Huntsville, United States. <sup>2</sup>Sam Houston University, Huntsville, United States

**Abstract**

*Staphylococcus aureus* is a common inhabitant of the skin and an upper respiratory tract in healthy individuals, but remains one of the most common causes of health-care related infections in the U.S. In this study, swabs of phone cases were swabbed and plated for bacterial growth. The 16srRNA gene was amplified and sequenced from a number of isolates to determine their identity. Four isolates identified as *Staphylococcus aureus* were sequenced using Oxford Nanopore Technology followed by genome assembly and annotation including multi-locus sequence typing and whole genome phylogenetic analysis.

## Assessing Pathogenicity of Wastewater-Derived Bacterial Isolates in *Caenorhabditis elegans*

Siyi Zhou, Seokju Seo, Kathryn Rhatigan, Marga Lee, [Alice Liu](#), Alyse Tran, Fernanda Vaca, Lois Armendariz, Todd Treangen, Natasha Kirienko, Lauren Stadler, Yousif Shamoo

Rice University, Houston, United States

### Abstract

Current environmental biosurveillance can track known biological threats but has significant limits for the detection of potential pre-emergent pathogens (PEPs). We developed a microfluidics-based Capture-Enrichment pipeline that preserves community diversity while enriching serum-tolerant cells from wastewater. The approach recovered PEPs that fell below the detection limits of conventional amplicon sequencing. Genomic analysis revealed wastewater-derived ESKAPE pathogens closely related to clinical isolates, showing that the pipeline captures clinically relevant threats. Furthermore, the captured PEP candidates exhibited phenotypes consistent with early-stage infection and antimicrobial resistance.

To determine whether these candidates were potentially pathogenic *in vivo*, we selected nine unusual isolates, varying in damage and persistence traits, for phenotypic characterization using a *C. elegans* infection model. In a Slow Killing assay, all nine isolates significantly reduced host survival with median lethal times ranging from 3 to 6 days, compared to 10 days for the *E. coli* control. Host-response reporters revealed distinct outcomes among isolates. Six isolates induced *irg-5p::GFP*, consistent with activation of infection-associated innate immune responses. One isolate increased DAF-16 nuclear localization, whereas several others favored cytoplasmic localization, indicating strain-dependent modulation of FOXO-associated stress responses. Three isolates induced *gst-4p::GFP*, consistent with activation of oxidative-stress and detoxification responses. These results show that wastewater-derived isolates can produce pathogenic phenotypes while eliciting distinct host-defense signatures.

Targeting PEPs can fundamentally improve biosurveillance by providing an early warning of potentially emerging microbial pathogens. This approach expands genomic databases that catalog functional sequences of concern, strengthening how PEPs are identified and characterized.

## Isolation and stress-tolerance assay of a potential dark septate fungal endophytes associated with the halophyte plant, *Batis maritima*

Linzee Pacheco, Candice Lumibao

Texas A&M University-Corpus Christi, Corpus Christi, United States

### Abstract

Root associated fungal endophytes play an important role in aiding plant tolerance to environmental changes and stressors, while plants provide shelter and nutrients. Dark septate endophyte fungi (DSE) are a polyphyletic, functional group of fungal endophytes that colonize the root cells of diverse plant hosts. DSEs have been shown to mediate physiological responses of plants to extreme environments such as in highly saline environments. However, knowledge on basic biology and ecology remains limited. In this study, root fungal endophytes were isolated from *Batis maritima*, a halophyte dominant in Oso Bay salt marshes in South Texas. Isolated endophytes were then genetically identified via DNA amplification and sequencing of the internal transcribed spacer (ITS) region, and from these, one potential DSE species (*Scolecobasidium musae*) was selected for salinity-stress assays to determine stress tolerance. The DSE strain was inoculated onto MEA containing a range of salinity concentrations (0ppt, 5ppt, 10ppt, and 20ppt) with seven replicates and allowed to grow for 84 days. Images were taken every week, and overall fungal growth (hyphal radial and colony growth) was analyzed using ImageJ. Results indicate that optimum growth occurred at the highest salinity level (20 ppt), suggesting that this endophyte species has high salt-tolerance, and possible most adapted to highly saline environment. Understanding how fungal endophytes adapt under saline conditions will allow for potential applications for agriculture such as growth promotion, soil health, and the aid of marsh ecosystem management.

**Time waits for no microbes: Higher isolation frequency and diversity of bacterial and fungal endophytes in modern vs. ancestral seeds of *Schoenoplectus americanus***

Trinity Storr, Candice Lumibao

Texas A&M University - Corpus Christi, Corpus Christi, United States

**Abstract**

Coastal marshes are highly productive ecosystems that support carbon storage, shoreline stabilization, and diverse biota. Along the Gulf and Atlantic coasts, marshes are significantly influenced by foundational species such as *Schoenoplectus americanus*. Marsh sediments can preserve *S. americanus* seeds for over a century, forming natural seed banks that allow comparisons between modern and ancestral plant populations. While plant traits and genetic variation change with sediment depth, little is known about whether seed-associated bacterial and fungal endophytes also vary with sediment depth. In this study, endophytes were isolated from *S. americanus* seeds from soil cores at two Chesapeake Bay marshes, Sellman and Corn Island. Stratigraphy was reconstructed using  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$ , with seeds sampled from 0–10cm (modern) and 16–30cm (ancestral). Seeds were surface-sterilized, sectioned, and cultured on nutrient (bacteria) or malt extract agar (fungi). Bacterial endophytes showed a higher isolation frequency (40%) than fungi (9%) across all seeds, the majority of which are from modern seeds. Modern seeds exhibited greater microbial diversity, including bacteria such as *Rhodanobacter*, *Curvibacter*, *Hydrogenophaga*, and *Pseudomonas*, as well as saprobic fungi such as *Helotiaceae* and *Aspergillaceae*. In contrast, ancestral seeds were dominated by stress-tolerant, spore-forming *Bacillaceae*, with *Bacillus* present at all depths, and showed limited fungal growth, with *Lulworthiaceae* as the sole fungal group recovered. Sellman seeds had greater overall diversity, while Corn Island seeds included some marine-related types. These results show that seed endophyte communities change over time, with modern seeds having more environmental microbes, while a stable internal microbiome remains across generations.

## **Salmonella S. Lethality Validation Model for Render Poultry Components**

Javier Zavala<sup>1</sup>, Matt Taylor<sup>2</sup>

<sup>1</sup>St. Edwards University, Austin, United States. <sup>2</sup>Texas A&M, College Station, United States

### **Abstract**

To meet U.S. safety standards, the commercial poultry industry must achieve a 7-Log<sub>10</sub> thermal reduction of *Salmonella enterica* in processed by-products. Historically, the industry relied on rigid log-linear models that fail to account for multivariate complexities like protein coagulation in chicken blood or container thermal conductivity. This project replaces these static mathematical equations with a dynamic machine learning approach to accurately forecast non-linear Salmonella survival curves. To overcome limited empirical data on chicken blood, datasets were supplemented with thermodynamic proxies like chicken broth and static USDA baselines to create a predictive "Data Cocktail." Data was standardized across six core parameters: time, temperature, container material, submersion level, and starting/final cell counts. Evaluated via Grid Search with five-fold cross-validation, XGBoost emerged as the optimal algorithm for mapping complex matrix interactions, achieving a testing R<sup>2</sup> of 0.9652. SHAP (SHapley Additive explanations) analysis confirmed the model successfully learned the underlying biological laws to overcome data scarcity, predicting survival outcomes where traditional linear models failed. This research successfully transitions predictive microbiology from rigid math to an adaptable learning model. Though some temperature data gaps remain, the resulting model is fully deployed as a full-stack web application, providing food safety professionals with an immediate, dynamic tool to predict pathogen lethality and ensure ongoing compliance.

## **Medical Microbiology Undergraduate Student Posters**

## Biodegradable Microsphere-mediated Phage Delivery to Treat Musculoskeletal Infections Caused by *Staphylococcus aureus*

Noemie Clemenceau<sup>1,2</sup>, Tanner Taylor<sup>1</sup>, Ambrose Catherine<sup>1</sup>, Heidi Kaplan<sup>1</sup>

<sup>1</sup>UTHealth / McGovern Medical School, Houston, United States. <sup>2</sup>Texas State University, San Marcos, United States

### Abstract

The rise of multidrug-resistant pathogens causing musculoskeletal infections (MSKIs) is a growing concern in orthopedic medicine. Here, we investigated the use of biodegradable chitosan microspheres for local delivery of anti-staphylococcus phage K as a potential treatment for multidrug-resistant musculoskeletal infections (MSKIs) caused by *Staphylococcus aureus*. In addition, we continued to phenotypically and genotypically analyze 64 contemporary *S. aureus* MSKI clinical isolates. Active phage K release from the microspheres was quantified using a double-layer plaque assay and measured over 20 days. We determined phage adsorption to the microspheres was high and that active phage was released gradually over time. By measuring hemolytic activity of the isolates on sheep blood plates, we found seven of the 64 clinical isolates were non-hemolytic. The genetic analysis placed the majority (54.6%) of the clinical isolates into *agr* group 1, 29.7% into group 2, and 15.6% into group 3 based on their *agrD* DNA sequences. Furthermore, the phage K receptor biosynthesis genes, which produce and modify wall teichoic acids, were screened for mutations, and a premature stop codon was identified within the *tarM* gene of MH49, which is resistant to all ten phage in our library. The chitosan microsphere elution results suggest that the microspheres will be ready for testing in our *in vivo* rat model of orthopedic device-related infections when their diameters are reduced to 20-50  $\mu$ M. This analysis of our clinical isolates highlights the need for further investigation into their differences.

## Differential Impacts of Growth Media and Sugar Sources on Urinary *Enterococcus faecalis* Biofilm Formation

Edidiong Cornel<sup>1</sup>, Ibrahim Salaudeen<sup>1</sup>, Nicole De Nisco<sup>1,2</sup>

<sup>1</sup>The University of Texas at Dallas, RICHARDSON, United States. <sup>2</sup>University of Texas Southwestern Medical Center, Dallas, United States

### Abstract

*Enterococcus faecalis* is a Gram-positive-bacterium and commensal of the human gastrointestinal (GI) tract. However, outside of the GI tract, *E. faecalis* is an opportunistic pathogen that causes complicated and recurrent urinary tract infections (UTIs). Biofilm formation contributes significantly to *E. faecalis* virulence during catheter-associated UTI, but its role in recurrent UTI is less well studied. Although nutrient availability has been implicated as a mediator of *E. faecalis* biofilm formation, systematic comparisons across different growth conditions and strains remain limited. To identify metabolic mediators of biofilm formation, we characterized biofilm production by genetically diverse *E. faecalis* strains isolated from blood, gastrointestinal, and urinary sources across different media types.

We tested biofilm formation by 60 *E. faecalis* strains in chemically defined medium with 0.5% glucose, fructose or mannose and artificial urine medium with no added sugar, 0.5% glucose or 0.5% mannose. Biofilm formation was measured after 24-hour incubation in round-bottom 96-well plates by crystal violet assay.

Overall, we saw large variations in biofilm formation across media types and strains with optical density values ranging from 0.07 to 5.68. Biofilm formation was generally higher in AUM compared to CDM conditions and was most significantly elevated in AUM with glucose or mannose, especially for urinary isolates. One urinary isolate, C14.2, demonstrated 4-fold higher biofilm formation in AUM mannose compared to AUM alone. The consistently elevated biofilm formation in AUM glucose compared to CDM glucose, suggests a component of AUM enhances biofilm formation. These patterns suggest that environmental metabolic cues may impact strain-specific biofilm phenotypes.

## Metabolic Diversity Among *Enterococcus faecalis* Isolates from Urinary and Gastrointestinal Niches

Chigemezu Eke, Ibrahim Salaudeen, Nicole De Nisco

University of Texas at Dallas, Richardson, United States

### Abstract

*Enterococcus faecalis* is a Gram-positive commensal of the gastrointestinal tract and an opportunistic pathogen frequently associated with urinary tract infections. Previous comparative genomic analysis identified 19 genes enriched among urinary *E. faecalis* isolates, including several involved in carbohydrate transport and metabolism, suggesting differences in metabolic capacity. We therefore hypothesized that urinary isolates would utilize a different range of carbon sources than gastrointestinal isolates. After conducting an initial Biolog carbon utilization phenotyping array on a subset of *E. faecalis* isolates, 10 carbon sources, including glucose as a universal growth control, were prioritized for further screening. We assessed the ability of 60 *E. faecalis* strains from urinary, gastrointestinal, blood, and oral sources to utilize sucrose, mannose, maltose, mannitol, fructose, ribose, trehalose, gluconic acid and galactose by evaluating endpoint growth in chemically defined medium supplemented individually with the necessary sugar as a sole carbon source.

Mannose, fructose, and maltose supported the highest overall endpoint growth of the tested strains, whereas several other substrates supported strong growth only in subsets of isolates. For example, five gastrointestinal isolates displayed robust growth on sucrose, whereas all urinary isolates demonstrated a limited ability to utilize sucrose as a carbon source for growth. Conversely, urinary isolate C130 showed the highest endpoint growth across all tested strains on maltose, mannitol, ribose, and trehalose. These findings demonstrate substantial strain-level metabolic diversity in *E. faecalis* and support further characterization of strain-specific metabolic differences and their roles in *E. faecalis* host colonization and pathogenicity.

## Spatial Patterns of the Oral Microbiome and Dental Disease in Patients with Low Salivary Flow

Michael Fowler<sup>1,2</sup>, Juiyun Tsai<sup>2</sup>, Diana Proctor<sup>2</sup>

<sup>1</sup>University of Houston Downtown, Houston, United States. <sup>2</sup>UT Health Houston, Houston, United States

### Abstract

Dental cavities, or caries, are caused by aberrant activity of the bacteria at the tooth surface. Salivary flow prevents caries through ion exchange-driven mineralization and oral clearance of sugars and acids. Low salivary flow, or hyposalivation, is associated with increased caries frequency and lesions at atypical sites, including anterior root and coronal surfaces. Here, we sought to evaluate the contribution of salivary flow to the spatial patterning of the oral microbiome and dental disease. Adults were enrolled into control (n=119) or low flow (n=32) cohorts. Tooth-specific linear models quantified associations between age, unstimulated whole salivary flow rate (UWS-FR), clinical attachment loss (CAL), and decayed, missing, or filled surfaces (DMFS). Lower UWS-FR was associated with increased DMFS and CAL at 21 teeth. Preliminary microbiome analysis of six participants revealed significant differences in community structure between cohorts, whereas age was an imperfect predictor. Survey data from 109 controls and 32 low flow participants were also analyzed for differences in lifestyle and dental behaviors. Participants in the low flow cohort flossed ( $p<0.03$ ), used mouthwash ( $p<0.01$ ), and visited the dentist more frequently ( $p<0.01$ ) than the control cohort, consistent with prior reports that patients with low flow engage in more intensive oral-health practices. Out of 39 dietary behaviors, only the chewing of xylitol gum ( $p<0.01$ ), nut consumption ( $p<0.01$ ), and soda consumption ( $p<0.05$ ) significantly differed between cohorts. Together, these results suggest reduced salivary flow is associated with spatially patterned dental disease and altered microbiome structure, despite more frequent preventative behaviors.

## Potential Antiviral Defense through G3BP-induced Stress Granule Formation

Kavin Gidvani

Texas A&M, College Station, United States

### Abstract

My project focuses on stress granules which are ribonucleoprotein assemblies that form in response to cellular stress. They are mainly regulated by the proteins G3BP1&2 which may support antiviral defense by concentrating on proteins involved in viral RNA detection such as G3BP. My project focuses on investigating this pathway by placing U2OS osteosarcoma and HEK293T human embryonic kidney cells under cellular stress using sodium arsenite ( $\text{NaAsO}_2$ ) and FAZ (a G3BP inhibitor). My results showed that cells receiving  $\text{NaAsO}_2$  and FAZ showed greater recovery than cells treated with  $\text{NaAsO}_2$  alone with negligible differences between cell lines. Although further research of stress granules and viral infection correlation are needed, these findings can improve our understanding of how G3BP mediated stress-granule regulation influences cellular antiviral responses.

## **Ethanol increases biofilm formation of gut- and bladder-associated pathogens *Escherichia coli* and *Enterococcus faecalis*.**

Sribhavya Koneti, Jacob Hogins, Larry Reitzer

University of Texas at Dallas, Richardson, United States

### **Abstract**

An estimated 400 million people, or 7% of the world's population, have dealt with alcohol use disorder at some point in their lives. Alcohol overuse is clinically linked to a fourfold increased risk for inflammatory bowel disease (IBD) and a 60% increased risk for recurrent urinary tract infections (UTIs). Two bacterial species correlate with these conditions: *Escherichia coli* (Ec) causes 85% of UTIs and co-occurs with *Enterococcus faecalis* (Ef) in a quarter of cases, and both species dominate the dysbiotic gut, where Ec drives inflammation linked to IBD. This study investigates how alcohol alters the behavior of these gut- and bladder-associated pathogens, specifically their capacity to form biofilms, a key driver of infection persistence and treatment failure. Ec-derived strains (K40 and IBD-derived clinical strains) and clinical Ef strain HRH40 were grown overnight. Pure single colonies were inoculated into a nutrient-rich medium, normalized to OD<sub>600</sub> 0.01 with or without 0.03% ethanol, and incubated in 24-well plates at 37°C overnight under aerobic and anaerobic conditions. Biofilm biomass was quantified by the crystal violet assay, and absorbance was measured at 570 nm. Ethanol exposure increased biofilming across Ec and Ef strains under both oxygen conditions, with the magnitude of induction varying by species, strain, and oxygen availability. Future work will examine biofilm formation with or without adrenergic acid (a host inflammatory signal increased in both UTI and IBD) to further explore associations with gut and bladder inflammation.

## Nutritional availability: Influence on polymicrobial interaction

Vicky Nieniel, Todd Primm, Aaron Lynn, Anand Karki

Sam Houston State University, Huntsville, United States

### Abstract

Polymicrobial interactions between bacterial species can influence their survival, growth, and ability to establish infections in animal hosts. *Salmonella* spp. and *Enterococcus* spp. are important bacterial species associated with food safety, and interactions between these organisms may contribute to their persistence and pathogenicity. This ongoing study investigates how variable nutrient compositions influence the interaction between *Salmonella enterica* and *Enterococcus faecalis* strain. Bacterial interactions are being examined in five nutritional environments: Mueller-Hinton Broth (MHB), Brucella Broth (BB), Phosphate-Buffered Saline (PBS), chicken juice (CJ), and Simulated Intestinal Fluid (SIF). Viable counts are being measured over 24 hours of incubation to determine changes in bacterial survival and growth over time. The study aims to determine whether nutrient availability influences polymicrobial interactions and to provide insight into how environmental conditions may affect *Salmonella/Enterococcus* survival and potential biofilm-associated infections in animal hosts.

## Examining the *Galleria mellonella* Gut Microbiome to Model *Campylobacter* Infection

Nikhil Patel, Samuel Cannon, Anand Karki, Todd Primm  
Sam Houston State University, Huntsville, United States

### Abstract

*Campylobacter jejuni* is the most common bacterial cause of foodborne gastroenteritis worldwide. In immunocompromised individuals, late manifestations of campylobacteriosis include autoimmune disorders such as Guillain-Barre Syndrome and its variant, Miller-Fisher Syndrome. The human gut microbiome is both dense and diverse, requiring oral pathogens to have effective invasion strategies. Therefore, current hypotheses suggest that *C. jejuni* forms relationships with specific gut bacteria to develop a biofilm or participates in horizontal gene transfer to create an infection. To examine such interactions, *Galleria mellonella* (wax moth) larvae were developed as a tractable model organism. The larvae can survive at human body temperature and their gut microbiomes are significantly less complex than the human gut microbiome. For these microbiomes with and without *Campylobacter*, DNA will be extracted, and the combined 16S rRNA gene content will be PCR-amplified. Oxford Nanopore sequencing will reveal larval community compositions and identifications. Collectively, a holistic understanding will be developed on how this species manipulates other gut bacteria to establish an infection.

## MURF2 Protects Against Viral Myocarditis by Promoting Type I Interferon Production

Ananya Pathak<sup>1</sup>, Junying Wang<sup>1</sup>, Wenting Lu<sup>1</sup>, Zhiqiang Zhang<sup>1,2</sup>, Junji Xing<sup>1,2,3</sup>

<sup>1</sup>Immunobiology and Transplant Science Center, Houston Methodist Academic Institute, Houston, United States. <sup>2</sup>Department of Surgery, Weill Cornell Medicine, Cornell University, New York, United States. <sup>3</sup>Department of Cardiovascular Sciences, Houston Methodist Academic Institute, Houston, United States

### Abstract

**Introduction:** Viral myocarditis, often caused by coxsackievirus B3 (CVB3), is a major cause of sudden death in young adults and can progress to dilated cardiomyopathy. Dysregulated innate immune responses contribute to cardiomyocyte injury and inflammation, yet effective therapies remain limited. We previously showed that the E3 ubiquitin ligases TRIM18 and TRIM29 regulate viral myocarditis pathogenesis. Given the high expression of muscle-specific RING finger protein 2 (MURF2) in cardiac macrophages, we hypothesized that MURF2 regulates antiviral immunity during CVB3-induced myocarditis.

**Methods:** We used in vivo and in vitro approaches to define MURF2's role in CVB3-induced myocarditis. Wild-type (WT) and MURF2 knockout (KO) mice were infected intraperitoneally with CVB3; survival, cardiac histopathology, viral titers, and type I interferon (IFN) levels were assessed. Bone marrow-derived macrophages (BMDMs) from WT and MURF2 KO mice were stimulated with dsRNA or infected with cardiotropic RNA viruses, and type I IFN production was measured by ELISA. Co-immunoprecipitation and immunoblotting examined the MURF2-IRF3 interaction and characterized IRF3 ubiquitination.

**Results:** MURF2 promoted type I IFN production following dsRNA stimulation and cardiotropic RNA virus infection. Compared with WT mice, MURF2 KO mice exhibited reduced survival, increased viral titers and inflammation, and diminished type I IFN responses after CVB3 infection. MURF2 interacted with IRF3 and promoted K63-linked ubiquitination of IRF3, enhancing IRF3 expression and restricting CVB3 replication.

**Conclusions:** These findings identify MURF2 as an important regulator of antiviral immunity, protecting against CVB3-induced myocarditis. The MURF2-IRF3 axis is a potential therapeutic target for enhancing antiviral defense and limiting virus-induced cardiac injury.

## **A Novel Murine Model of Polymicrobial UTI Reveals Disrupted Group D *Escherichia coli* Growth in *Enterococcus faecalis* Colonized Bladders**

Carter Pruitt<sup>1</sup>, Satya Vedula<sup>1</sup>, Waris Khuwaja<sup>1</sup>, Jacob Hogins<sup>1</sup>, Phillipe Zimmern<sup>2</sup>, Larry Reitzer<sup>1</sup>, Xintong Dong<sup>1</sup>

<sup>1</sup>The University of Texas at Dallas, Richardson, United States. <sup>2</sup>The University of Texas Southwestern Medical Center, Dallas, United States

### **Abstract**

Polymicrobial urinary tract infections (UTIs) pose emerging threats to standard UTI treatment, with patients often experiencing increased complications and recurrent infections. While uropathogenic *Escherichia coli* (*Ec*) strains typically belong to phylogenetic group B2, phylogroup D strains account for roughly 25% of *Ec* infections and often co-isolate with *Enterococcus faecalis* (*Ef*) in clinical samples. Evidence from our lab showed that *Ef* co-cultured with group D *Ec* results in large transcriptome alterations, suggesting interspecies interactions in polymicrobial uropathogenesis. However, a reliable animal model of polymicrobial UTI has yet to be described to investigate these findings *in vivo*.

Here, we developed a murine model of acute polymicrobial cystitis and examined the effect of *Ef* on *Ec* virulence factor synthesis *in vitro*. Using a sequential dual-*Ef* inoculation approach, we describe the first known protocol for reliably establishing *Ef* colonization in a murine bladder. Secondary *Ec* inoculation following *Ef* colonization unexpectedly showed significantly diminished *Ec* titers compared to *Ec*-monoinfected controls. These results demonstrate that *Ef* colonization restricts the growth of group D *Ec* in a host environment, which may consequently stimulate virulence factor synthesis and stabilize colonization in the bladder. *In vitro* results showed that *Ec* cultured in *Ef*-conditioned media increased expression of two key virulence factors: type-1 fimbriae as assessed by yeast agglutination and hemolysin as assessed by lysis of erythrocytes. By establishing the first animal model for acute polymicrobial infection, this work delivers a novel platform to investigate microbial interactions at the tissue-level, providing crucial insights into the pathogenesis of polymicrobial UTIs.

## Transcription factors MRR1 and MRR2 affect biofilm growth in *Candida albicans* CAUTI infections

Conner Schultz, Shane Cristy, Michael Lorenz, Pedro Miramon Martinez

UT Health Houston, Houston, United States

### Abstract

*Candida albicans* is an opportunistic fungal pathogen responsible for the most nosocomial infections worldwide. It is the second most common cause of Catheter-Associated Urinary Tract Infections (CAUTIs) in the United States, yet little is known about its colonization pathway in the urinary tract. CAUTI Biofilms exhibit increased resistance to the antifungal Fluconazole, likely due to upregulation of xenobiotic detoxification mechanisms, such as efflux pumps. Transcription factors related to efflux pumps, such as MRR1, have been shown to be required for CAUTI biofilm growth and polymorphisms provide a fitness advantage in urine. Here, we explore the effects of gain-of-function and loss-of-function polymorphisms in MRR1 to better understand its contribution to CAUTI infections, and investigate the importance of MRR2, a silicone adherence regulator, in silicone catheters. We found that while LOF and some GOF MRR1 polymorphisms had no significant impact on CAUTI biofilm growth in vitro, polymorphisms that impacted the inhibitory domain of the protein increased it. This suggests that inhibition of MRR1 hinders both biofilm growth and fluconazole resistance in CAUTI environments. Future research is needed to understand the regulatory mechanisms of MRR1 and to identify potential promoters. Deletions of MRR2 also have no significant impact on biofilm growth in latex CAUTI environments or on fluconazole; the effects of MRR2 polymorphisms in CAUTI *C. albicans* isolates are still unknown.

## Impact of mucin on the virulence of *Campylobacter jejuni*.

Brianna Smith, Killian Makin, Samuel Cannon, Anand Karki, Todd Primm

Sam Houston State University, Huntsville, United States

### Abstract

*Campylobacter jejuni*, a Gram-negative microaerobic foodborne pathogen, thrives in mucosal gut environments, most notably in the Gastrointestinal tract of chickens and humans. While colonizing but not causing over harm in chickens, in contrast, *C. jejuni* in humans is the leading cause of bacterial gastroenteritis. Part of the immune defenses in the intestine is mucus, which is primarily made of mucins. Mucins are heavily-glycosylated proteins which are very hydrated and often cross-linked using disulfides. Mucins form a sticky net which captures invading microbes, preventing access to gut epithelial surfaces and blocking invasion and attachment. We propose that mammalian mucins may be a key signal for the bacteria to activate virulence factors, which could help explain the difference between mildly damaging chicken colonization compared to pathogenic human infection. Exposure of *C. jejuni* in vitro to purified pig mucin may result in an increase in the growth and virulence of the *C. jejuni* when compared to a sample that was not exposed to purified pig mucin. According to preliminary data, there was a sharp increase in *C. jejuni* growth after exposure followed by a period of death and then stabilization of *C. jejuni* growth, suggesting that pig mucin supports virulence factors in *C. jejuni*.

## Genetic Reversion to Validate *Streptococcus agalactiae* Pullulanase (SAP) as a Virulence Factor in Group B Streptococcus

Sneha Chandrashekar, Krishi Chevendra, Cody Cutts, Bryan Estrada, Brandon Kim  
University of Texas at Dallas, Richardson, United States

### Abstract

*Streptococcus agalactiae* (Group B Streptococcus, GBS) is a gram-positive commensal of the human intestinal and female genitourinary microbiota that causes neonatal sepsis, pneumonia, and meningitis, remaining the leading cause of neonatal meningitis worldwide. To identify GBS genes involved in this process, we profiled gene expression during infection of a stem cell-derived brain endothelial cell (iBEC) model using dual RNA sequencing and identified *Streptococcus agalactiae* Pullulanase (SAP) as highly upregulated during infection. A SAP knockout mutant showed significantly reduced adherence to and invasion of iBECs, along with decreased tight-junction barrier disruption.

To determine whether this change was caused by SAP, and rule out off-target effects, we applied Molecular Koch's postulates, which require gene loss, reversion, and phenotype restoration to establish causality. Our approach uses CRISPR-Cas12 to reintroduce functional SAP into the knockout mutant, generating a back-to-wild-type (BTWT) strain. A guide RNA and repair cassette containing wild-type SAP flanked by homology arms were assembled with an aTc-inducible Cas12 expression cassette into a single plasmid via Gibson assembly. The construct was transformed into DH5α *Escherichia coli* by heat shock and screened by colony PCR/gel electrophoresis for the expected 4,539 bp product before purification via midiprep.

Current work addresses genomic DNA carryover limiting plasmid concentration by adjusting culture conditions, reducing temperature and antibiotics, for cleaner extractions, and by transforming directly into GBS to avoid degradation by native restriction-modification systems. Restoration of wild-type adhesion and invasion would confirm SAP's contribution to GBS neuroinvasion and the utility of CRISPR-Cas12 complementation for studying GBS virulence factors.

## **General Microbiology Graduate Student Posters**

## Investigation of dUTPase as a Novel Iron Acquisition Enzyme in *Bacillus anthracis*

Adewale Adewole<sup>1</sup>, Kyle Gallegos<sup>1</sup>, Sophie Degrand<sup>1</sup>, Gracie Miroballi<sup>2</sup>, Jessica Guilhas<sup>1</sup>, Shauna M. McGillivray<sup>1</sup>

<sup>1</sup>Texas Christian University, Biology Department, Fort Worth, United States. <sup>2</sup>Texas Christian University, Fort Worth, United States

### Abstract

Deoxyuridine triphosphatase (dUTPase) is a critical and often essential enzyme which ensures genome integrity by hydrolyzing deoxyuridine triphosphate (dUTP) to deoxyuridine monophosphate (dUMP). Surprisingly, we identified the dUTPase gene as necessary for acquiring iron from hemoglobin during a transposon mutagenesis screen in *Bacillus anthracis* Sterne under iron-limiting conditions. dUTPase has never been linked to iron acquisition in any species, however some dUTPases have been reported to possess additional regulatory functions outside dUTP hydrolysis, including transcriptional regulation. We hypothesize that *B. anthracis* dUTPase contributes to iron acquisition by regulating the expression of genes involved in iron acquisition, potentially through interactions mediated by its distinct structural domains, the catalytic domain and variable domain. The goal of this study is to determine which dUTPase structural domains contribute to iron acquisition and survival under iron-limiting conditions. Preliminary findings demonstrate that the dUTPase transposon mutant (9f12tn) exhibits impaired growth and survival under iron-limiting conditions, while genetic complementation restores these phenotypes. Current work aims at introducing mutation into the catalytic domain of dUTPase to determine whether dUTP hydrolysis is necessary for iron acquisition from hemoglobin and survival under iron-limiting conditions. Ultimately, this work will provide insight into a previously unrecognized role for dUTPase in bacterial pathogenesis and may reveal a novel mechanism by which *B. anthracis* adapts to nutrient limitation during infection.

## Enterococcus & Salmonella: Bacterial Competition and/or Co-existence

Marbella Canaca, Todd P. Primm, Aaron Lynne, Anand Karki

Sam Houston State University, Huntsville, United States

### Abstract

*Salmonella enterica* is a major foodborne pathogen commonly associated with raw retail poultry, along with other foodborne pathogens such as *Campylobacter jejuni*. *Enterococcus* spp., including *Enterococcus faecalis*, are also commonly found in coexistence and recovered from the gastrointestinal microbiota of poultry. *Enterococcus* spp. has proven to be extremely resilient, out-competing co-inhabiting bacteria through production of metabolites, such as enterocin, surviving a wide range of environments, and contributing towards antimicrobial resistance. *E. faecalis* is a growing challenge as an opportunistic pathogen in clinical settings. However, its resilience and competitiveness have also demonstrated some benefits, such as inhibiting the growth of other harmful pathogens through competitive exclusion. This study aims to investigate the polymicrobial interactions between *Enterococcus faecalis* and *Salmonella enterica* in varying stress conditions that mimic the human gut, such as low pH, bile salt tolerance, and biofilm formation to better understand the role *E. faecalis* plays in the co-cultures. Additionally, production of metabolites will also be tested to help determine the influence they have on the growth of *S. enterica*. It is hypothesized that in co-culture, *E. faecalis* may inhibit the growth of *S. enterica* in the various assays.

## Temperature and Oxygen Availability Shape Pel-Dependent Biofilm Architecture in *Pseudomonas aeruginosa* PA14

EMMANUELLA ASIAMAH, Catherine Wakeman

Texas Tech University, Lubbock, United States

### Abstract

*Pseudomonas aeruginosa* forms biofilms that promote persistence across diverse environmental and host-associated conditions. However, changes in temperature and oxygen availability may alter biofilm architecture and the contribution of specific extracellular polymeric substances (EPS). This study investigated how temperature, oxygen availability, and Pel polysaccharide influence biofilm formation in *P. aeruginosa* PA14 as a foundation for examining microplastic-associated bacterial colonization.

PA14 wild type and a Pel-deficient mutant ( $\Delta$ pel) were grown under static conditions at 23°C and 37°C under aerobic and reduced-oxygen environments. Biofilm formation was evaluated as pellicles at the air-liquid interface and as submerged surface-associated biofilms on glass. At 23°C, wild-type PA14 produced robust pellicle biofilms, whereas at 37°C there was reduced pellicle formation and promoted surface-associated growth. The  $\Delta$ pel mutant displayed impaired pellicle architecture but retained substantial surface-associated biofilm formation, particularly at 37°C. Oxygen availability further modified these phenotypes, demonstrating that biofilm architecture depends on interactions among temperature, oxygen, and matrix composition.

Confocal fluorescence microscopy further revealed differences in biomass organization and EPS-associated signals between wild type and  $\Delta$ pel biofilms. Wild-type biofilms generally maintained greater structural continuity, whereas  $\Delta$ pel biofilms exhibited increased fragmentation and architectural heterogeneity across environmental conditions. Together, these findings demonstrate environmentally dependent biofilm transitions in PA14 and suggest that Pel contributes to biofilm organization rather than uniformly determining total biofilm formation. These results establish experimentally defined conditions for future studies testing whether polystyrene microplastics provide surfaces that facilitate *P. aeruginosa* attachment, biofilm development, and persistence in mosquito-associated environments.

## Fe-sistance: The Impact Of Iron Memory On Antibiotic Resistance In *Escherichia coli*

Marta Cano Minarro, Souvik Bhattacharyya

UTHealth Houston Science Center, Houston, United States

### Abstract

Bacteria have evolved to exhibit adaptive behaviors that resemble memory, in which past life history influences future behaviors, preparing cells for future encounters and reducing decision time. *Escherichia coli* can store information in the form of heritable intracellular iron states, a phenomenon termed 'iron memory', impacting flagellar motility on surfaces across generations. Because iron metabolism in *E. coli* is linked to many core physiological processes, including energy metabolism, transport, envelope, and redox homeostasis, heritable iron states likely carry information that enables adaptation to diverse environmental challenges, such as antibiotic exposure. Because bactericidal antibiotics are known to induce reactive oxygen species (ROS), intracellular iron may amplify ROS-mediated damage through Fenton chemistry, suggesting that lower iron levels could promote survival. Therefore, we hypothesize that low intracellular iron states confer increased survival against bactericidal antibiotics and that their heritability extends this protective phenotype across generations. Here we show that *E. coli* cells with lower iron levels survive better against multiple bactericidal and even bacteriostatic antibiotics, suggesting that low iron states influence antibiotic survival through mechanisms beyond redox homeostasis. A high-resolution iron reporter revealed cell-to-cell heterogeneity in iron levels, with a subpopulation entering low iron states under antibiotic stress. These low iron states were transiently retained after antibiotic removal, demonstrating time-dependent information retention consistent with cellular memory. We propose a model in which these iron memory states shape cellular metabolism and envelope homeostasis, shaping antibiotic survival.

## Regulation of Secretion Systems in *Paraburkholderia* During *Dictyostelium discoideum* Infection

Nazmul Rahman Chowdhury, Margaret Steele

Texas Tech University, Lubbock, United States

### Abstract

Intracellular bacteria, whether pathogenic or symbiotic, must overcome major challenges by manipulating the host environments to survive and replicate inside the host. Many of them use secretion systems to deliver proteins directly into host cells to alter their behavior. The relationship between the amoeba *Dictyostelium discoideum* and its three *Paraburkholderia* symbionts (*P. agricolaris*, *P. hayleyella*, and *P. bonniea*) provides an opportunity to study both mutualistic and pathogenic interactions. Although all three symbionts possess conserved T3SS and T6SS gene clusters, they don't behave the same way during infection. This raises key questions about when these secretion systems turn on during infection and how their regulation varies among the three species. We hypothesize that these secretion systems are essential for infection, and that differences in their regulation may explain differences in infection prevalence. To address this, we are establishing the molecular framework necessary to construct promoter-GFP reporter plasmids and future gene-deletion tools in *Paraburkholderia*. This includes identifying upstream regulatory regions of T3SS and T6SS genes and cloning the upstream regulatory regions of secretion system genes into GFP reporter plasmids, enabling fluorescence-based detection of gene activation during infection. Eventually, we will allow these clones to infect *D. discoideum* and use time lapse microscopy to measure secretion system activity during infection. This foundational work will enable precise monitoring of secretion system gene expression and support the development of genetic tools for dissecting host-microbe interactions in this emerging symbiotic model.

## Structure-Guided Phage Engineering Insights from the Structure of Mycobacteriophage Ryadel Before and After DNA Ejection

Faith Cox<sup>1</sup>, Michael Sherman<sup>1</sup>, Elena Orlova<sup>1</sup>, Dustin Edwards<sup>2</sup>, Thomas Smith<sup>1</sup>

<sup>1</sup>University of Texas Medical Branch, Galveston, United States. <sup>2</sup>Tarleton State University, Stephenville, United States

### Abstract

Phage therapy is a promising therapeutic for nontuberculous mycobacterial infections, but rational engineering is hindered by a lack of high-resolution structures. We present the cryo-EM structures of the mycobacteriophage Ryadel, before and after DNA ejection. The prolate capsid, determined to 7Å, is fully coated with polyglycans that could dampen immune recognition in clinical applications. Reconstruction of the neck region at 2.57Å, shows a novel interaction between the capsid and portal proteins that overcomes the Achilles' heel of tailed bacteriophage virions: the capsid opening at the symmetry transition between the 5-fold capsid and the 6-fold neck proteins. The N-termini of 5/12 portal proteins extend outside the capsid and line the entire capsid opening, facilitating the symmetry transition that allows the tail to attach to the capsid. Reconstruction of the tail tip before and after DNA ejection was determined to 2.89 and 3.1Å, respectively. Before DNA ejection, the baseplate-associated protein (BAP) at the tail tip encases the remaining tail-tip proteins that complete the DNA delivery channel. Following DNA ejection, each protein in the tail tip undergoes conformational changes. The BAP explodes outward and no longer secures the tail-tip proteins. The distal tail protein moves down, parallel to the bacterial membrane, and the baseplate hub protein opens the DNA delivery channel. The tail fiber protein is absent from the post-ejection structure. These high-resolution structures of a siphovirus baseplate transition, symmetry mismatch compensation, and glycan shielding provide insights for structure-guided, rational phage engineering therapy against nontuberculous mycobacteria.

## Exploring the Impact of Simulated Microgravity on *Campylobacter jejuni* and Mixed-Species Cultures

Kyleigh Howard, Joselyn Vazquez-Sanchez, Anand Karki, Madhusudan Choudhary  
Sam Houston State University, Huntsville, United States

### Abstract

The foodborne pathogen *Campylobacter jejuni* is one of the leading causes of gastroenteritis worldwide and is commonly spread through raw, contaminated poultry. The biological processes and responses of *C. jejuni* are vastly known in normal gravity conditions, but little is known about how this bacterium is affected under either simulated microgravity conditions or during spaceflight. Several bacterial species are influenced by their growth-kinetics, cellular processes, increased virulence, and antimicrobial resistance properties. This study analyzes the effects of simulated microgravity on the growth, morphology, and gene expression patterns of a single species culture of *C. jejuni* and the mixed species cultures of *C. jejuni* with other bacterial species. Preliminary data shows that there is no significant difference in the growth of *C. jejuni* between the simulated microgravity and normal gravity conditions. We are currently examining the gene expression patterns under both conditions, and it is anticipated that there will be transcriptomic changes in the *C. jejuni* and that microgravity will influence the interactions between *C. jejuni* and other bacterial species in the mixed culture environment.

## Generation and Characterization of a Genetically Stable mScarlet-Expressing Group B Streptococcus Strain Using CRISPR/Cas12a-Mediated Chromosomal Knock-In

Aryah Lee, Ashley McDonald, William Cutts, Brandon Kim  
University of Texas at Dallas, Richardson, United States

### Abstract

*Streptococcus agalactiae* (Group B Streptococcus, GBS) is a Gram-positive opportunistic pathogen and leading cause of neonatal meningitis worldwide. Despite its clinical burden, no vaccine is available, highlighting the need to understand GBS pathogenesis. Fluorescent bacterial strains enable visualization of localization and migration during infection; however, stable chromosomal fluorescent reporters in GBS remain limited. We hypothesized that targeted CRISPR/Cas12a-mediated integration of *mScarlet* into the GBS chromosome would produce stable fluorescence without altering bacterial growth or host-cell interaction phenotypes. Our objective was to generate and characterize an mScarlet-expressing GBS strain for visualizing bacterial migration during infection and establish a platform for future genetic manipulation of GBS. A plasmid-mediated CRISPR/Cas12a genome-editing strategy achieved chromosomal integration of *mScarlet*, with Cas12a and guide RNAs regulated by a tetracycline-inducible promoter and a homology-directed repair construct facilitating integration. PCR-based molecular screening confirmed targeted *mScarlet* insertion, and fluorescence microscopy confirmed fluorescence of the GBS-mScarlet strain. Growth-curve analyses revealed no significant growth defect following integration. Flow cytometry will further characterize mScarlet expression relative to wild-type GBS, with measurable fluorescence changes expected. Cell-based adherence and invasion assays will evaluate effects on host-cell interactions. Genomic insertion provides stable fluorescence without plasmid maintenance or antibiotic selection during replication. These findings establish CRISPR/Cas12a-mediated genome editing as a strategy for generating stable fluorescent GBS strains that support additional fluorescent markers, targeted gene deletion, and complementation. The COH1-mScarlet strain will enable visualization of bacterial migration while minimizing limitations of plasmid-based fluorescence, expanding the genetic toolkit for studying GBS virulence and host-pathogen interactions.

## Regulation of Methanol Metabolism in *Paracoccus denitrificans* through a Lanthanide Switch.

Prachi Mathur, Marcus Tsai, Trusha Parekh, Stephen Spiro

The University of Texas at Dallas, Dallas, United States

### Abstract

*Paracoccus denitrificans* can use one-carbon (C1) compounds such as methanol as a sole source of carbon and energy. Methanol is oxidized by a calcium-dependent methanol dehydrogenase (MDH) or a lanthanum-dependent enzyme (XoxF). In some methylotrophs, like *Methylobacterium extroquens*, *xoxF* and *mxoF* expression are reciprocally regulated by the presence or absence of lanthanum ions, with the XoxF protein acting as the lanthanum ion sensor. To study the lanthanide switch in *P. denitrificans*, we made in-frame gene deletions of *mxoF* and *xoxF*. We observed that the *mxoF* mutant can grow on methanol only in the presence of lanthanum ions, suggesting that XoxF provides an alternative route for methanol oxidation. Interestingly, the *xoxF* mutant shows a growth defect on methanol in both the presence and absence of lanthanum ions. Additionally, an *mxoF* promoter-*lacZ* reporter fusion shows that the *mxoF* promoter is switched off upon addition of lanthanum to methanol medium. Combined, these data suggest that the XoxF protein is required for growth on methanol and that it can alter the expression of *mxoF* in response to the presence of lanthanum. We present a model for the mechanism of the lanthanide switch in *P. denitrificans* which is based on these results and prior literature and extrapolates from studies of the switch in other methylotrophs.

## **Phi6 under (selective) pressure: driving viral evolution through exposure to varying relative humidities**

Laurissa Miller, Akiko Kaitlin Balitactac, Eric Bruning, Lisa Bono

Texas Tech University, Lubbock, United States

### **Abstract**

Only a fraction of all microorganisms, including viruses, are culturable; one of the abiotic factors influencing this phenomenon is relative humidity (RH). Factors such as RH and temperature are known to affect enveloped viruses. The bacteriophage phi6 is used as a model to study enveloped viruses. In this study, we exposed samples of phi6 to different RH percentages and temperatures for different periods of time. We found that exposing 1uL lysate droplets to 25°C at 10%, 80%, or 100% RH for 2 hours had the largest impact on viral survival and evaporation of the droplets out of all the different tested conditions. We then evolved phi6 over a 20-day period, exposing the samples to either 10%, 80% or 100% humidity at 25°C for 2 hours each day. We quantified the titer by plaque assay at the end of each incubation. We found that phage exposed to 10% and 100% RH had an initial decrease in titer with subsequent recovery. Titters exposed to 100% RH recover quicker than titers exposed to 10% RH, whereas all populations exposed to 80% RH went extinct. Moving forward, we plan to perform a paired growth assay (PGA) to measure the fitness of the evolved endpoints relative to the ancestor (WT). Then, we will sequence the endpoint lineages to determine pattern wise changes within the genomes relative to the WT. Our results show that RH is a strong selective pressure for enveloped viruses, but they can adapt to certain RH conditions.

## Determining host tropism of isolated *Klebsiella pneumoniae* phages

Tessa Nester, Alexandra Northcutt, Michael Allen

University of North Texas Health Science Center, Fort Worth, United States

### Abstract

Phage characterization is an essential step in the development of phage therapeutics. The objective of this study is to isolate novel phages against *Klebsiella pneumoniae* capsule type 2 and determine their host ranges and depolymerase activities. We used ATCC carbapenemase detection panel in liquid cultures and agar overlays to assess the host range and depolymerase activity of our phages. Phages HLW1 would only plaque on its original isolation host but killed from without on an additional three strains, demonstrating halos of depolymerase activity. HLW2 showed similar results to HLW1; however, it infected one additional strain. HAW2 demonstrated the broadest host range and infected four additional strains to the isolation host, including at least two capsule types while failing to show depolymerase activity against remaining strains of unknown capsule type. *K. pneumoniae* phages typically exhibit narrow host ranges, infecting strains of only one or two capsule types. Phage isolates HLW1 and HLW2 validate this, whereas phage HAW2 infected five strains including at least two capsule types. This shows superior potential for use in phage therapy and is supported by literature characterizing *K. pneumoniae* phages with larger host ranges. Genome analysis of our phages will further characterize host tropism and depolymerase potential. Additionally, phylogenetic analysis of bacterial strains will complete the picture of our phages host ranges.

## Effects of Gene Regulation of D-alanine Aminotransferase (*dat*) on *Staphylococcus aureus* Nasal Colonization

Samantha Ritter<sup>1</sup>, Katia Quintanilla<sup>1</sup>, Andrea Boyd<sup>1</sup>, Isabel Fernández-Escapa<sup>1</sup>, Katherine Lemon<sup>1,2</sup>

<sup>1</sup>Baylor College of Medicine, Houston, United States. <sup>2</sup>Texas Children's Hospital, Houston, United States

### Abstract

Nasal colonization by *Staphylococcus aureus* predisposes individuals to *S. aureus* infections at distal body sites. Defining factors that facilitate *S. aureus* colonization of the nasal epithelium can reveal new targets for decolonization therapy. Our lab recently showed that the *dat* gene is important for colonizing a nasal mucosal surface. We model *S. aureus* colonization with human nasal epithelial organoids differentiated at an air-liquid interface (HNO-ALI), which have apical mucus in contact with air. A transposon insertion in the *dat* gene has a  $\geq 1000$ -fold fitness defect in competition with the parental strain on HNO-ALI. Dat is one of two enzymes that synthesize the D-alanine required for cell wall integrity. Our data show Dat is the primary source of D-alanine on HNO-ALI, whereas it is relatively dispensable in culture media. The genetic regulation *dat* remains unknown. Complementation with *dat* under control of a putative inter-operon promoter region ( $P_{dat}dat$ ) restored the *dat*::Tn mutant to near parental levels on HNO-ALI, but not in defined medium lacking L-alanine. In contrast, complementation with *dat* under the main operon promoter ( $P_{pepV}$ ) rescued the *dat*::Tn mutant in both environments. This suggests that there is environment-specific regulation of  $P_{dat}dat$ . We hypothesize that nutritional constraints or environment-specific factors in the human nasal mucus layer regulate  $P_{dat}$ , contributing to colonization fitness. To investigate this, we will identify the promoter within the  $P_{dat}$  region and determine environmental factors affecting its regulation. This will improve understanding of *S. aureus* nasal colonization fitness and inform future Dat-targeted therapeutics.

## **Environmental Microbiology Graduate Student Posters**

**Predation resistance and responses to predator cues in *Pseudomonas* species associated with the bacterivorous soil amoeba *Dictyostelium discoideum* require the GacA regulon**

Margaret I. Steele<sup>1,2</sup>, Fahadul Alam<sup>2</sup>, Jessica M. Peiser<sup>3</sup>, Kaitlyn Torack<sup>1</sup>, Jacinta Otero<sup>2</sup>, Simon P. M. Dawson<sup>1</sup>, David C. Queller<sup>1</sup>, Joan E. Strassmann<sup>1</sup>

<sup>1</sup>Washington University in St. Louis, St. Louis, United States. <sup>2</sup>Texas Tech University, Lubbock, United States. <sup>3</sup>Columbia University, New York, United States

**Abstract**

Predation is likely to influence both the functioning of bacterial communities and the evolutionary process of pathogenicity in bacteria, since many of the characteristics that allow bacteria to evade predation are also those which can be exploited for biological control against plant pathogens, virulence, etc., or bioremediation. Many soil bacteria have evolved mechanisms to resist predation by protists such as *Dictyostelium discoideum*. Resistance appears to have evolved independently in some strains. Screening of over 6000 transposon mutants identified GacA as essential for resistance to *D. discoideum* predation in three strains of *Pseudomonas* and additional determinants of resistance in one strain included secondary metabolite biosynthesis genes and the exoprotease AprA. Transcriptomic analysis revealed variability between GacA regulons among strains with deletion of *gacA* significantly reducing transcriptional responses to supernatant from predators in two strains. The exception is *P. protegens*, which showed little WT transcriptional response to predator supernatants. Apart from the shared requirement for GacA, predation resistance mechanisms showed limited overlap among *Pseudomonas* species, with no universal role for AprA. GacA may be widely required for predation resistance in *Pseudomonas* through its positive regulation of secondary metabolism, secretion systems, and exoprotease production, together with its essential role in predator cue responses in some species.

## Isolation of biocement-producing bacteria for urea-free marine applications

Jennifer Bracewell, Kelli Palmer

University of Texas at Dallas, Richardson, United States

### Abstract

Manual intervention for concrete repair and replacement comes at high environmental and economic costs. Biocement, which can be formed by bacteria via microbially-induced carbonate precipitation (MICP), is a sustainable method for concrete repair. Biocement-forming bacteria can be incorporated into the concrete at mixing and then heal cracks where and when they occur. Biocement is not intentionally made by bacteria; rather, it is a byproduct of alterations to the local environment that occur during their normal metabolic activities. Bacteria thus “make” biocement by different metabolic mechanisms, and the environment plays a substantial role in the yield and physical properties of the biocement produced by a given bacterium. In particular, the marine environment is relatively understudied for biocement applications, yet there is a need for self-healing structures in this environment, wherein urea and nitrogenous waste, which are byproducts of using the most common model organism for MICP, would be detrimental to native biota. Here, we designed an enrichment protocol to isolate and characterize non-urea-requiring biocement-forming bacteria from Atlantic seawater. We identified three isolates, from the *Sulfitobacter*, *Marinobacter*, and *Bacillus* genera, two of which yielded higher biocement yields in seawater-mimicking media compared to non-ureolytic bacteria utilized in prior literature. Moreover, scanning electron microscopy (SEM)/energy dispersive spectroscopy (EDS) revealed distinct chemical and structural features of the biocement produced by bacteria in seawater-mimicking medium. Overall, our work establishes a pipeline for the isolation and characterization of novel biocement-forming bacteria from marine samples, with potential for application to marine self-healing materials.

## **Impacts of a common antibiotic, sulfamethoxazole, on stream biofilm structure and function**

Sarai Castro, Allison Veach

The University of Texas at San Antonio, San Antonio, United States

### **Abstract**

Biofilms are a matrix of microorganisms, proteins, and extracellular polymeric substance that serve as the most biologically active habitat in riverine ecosystems. Antibiotic pollutants entering rivers not only reduce water quality but may be proliferated in biofilms due to antibiotic resistance which can be propagated in biofilm matrices. Here, we performed initial field surveys to detect a common antibiotic discharged via wastewater treatment plants and septic tank leakage – sulfamethoxazole (SMX). This antibiotic is detected in rivers often because it isn't fully metabolized after consumption nor degraded after wastewater treatment. Further, antibiotic resistance genes (ARGs) corresponding to SMX are commonly detected in biofilm bacteria with higher levels being downstream from WWTPs. Future work for this study will include performing laboratory incubations with exposing field-grown biofilms (1 river site with low SMX concentration less impacted by wastewater and 1 site with high SMX concentrations impacted by wastewater) to a short-term, pulse exposure of high sulfamethoxazole concentrations in laboratory aquariums. Biofilm extracellular enzyme production for C and N degrading enzymes, quantitative PCR for detecting of bacterial abundance and sul-1 and sul-2 ARGs, 16S rRNA gene amplicons for biofilm microbiomes, and algal biomass will be estimated after destructive harvesting of biofilms. This work is essential for predicting how antibiotic pollution mechanistically impacts ARG proliferation and microbial biodiversity.

## Evaluating wild pig fecal pollution in coastal recreational and oyster-harvesting waters

Lydia Cates, Nora Bleth, Nicole Powers, Sam Sugarek, Jeffrey Turner  
Texas A&M University-Corpus Christi, Corpus Christi, United States

### Abstract

Invasive wild pigs are found across Texas with an estimated 2.6 million population size. While detrimental ecological impacts of feral swine are well documented in terrestrial environments, marine waters used for recreation and mariculture remain unassessed. Wild pigs are known carriers of many different viral and bacterial pathogens that can infect humans; fecal pollution originating from these feral swine can harbor these pathogens in marine waters and contribute to the overall health risk for recreators as well as emerging oyster mariculture operations. We hypothesized that wild pig fecal pollution decreased water quality and adversely affects oyster mariculture. To test our hypothesis, water quality has been assessed over the course of a 12-month sampling period (January 2025-December 2025) along the Aransas River and Copano Bay. The assessment involved measures of water quality and fecal pollution. Water quality was assessed through measurement of environmental conditions, nutrient concentrations, and fecal indicator bacteria concentrations (*Escherichia coli* and Enterococci). The presence and abundance of swine-specific fecal pollution is currently being determined by microbial source-tracking (MST) using the Pig2Bac PCR marker. Preliminary results show fecal indicator bacteria concentrations as *Escherichia coli* having a mean of 292 MPN (most probable number) and Enterococci having a mean of 61.21 MPN, both averaged across all sites and through the sampling period. Early ddPCR results show trace amounts of wild pig fecal pollution detected in water and oyster samples collected with ongoing analyses expected to contribute further support and complete results expected soon.

## "Exploring Microbial Water Quality in the Baffin Bay-Upper Laguna Madre: Preliminary Enterococci Findings"

Isabella Cevallos<sup>1</sup>, Michael Wetz<sup>1,2</sup>, Jeffrey Turner<sup>1</sup>

<sup>1</sup>Texas A&M University-Corpus Christi, Corpus Christi, United States. <sup>2</sup>Harte Research Institute, Corpus Christi, United States

### Abstract

The Baffin Bay-Upper Laguna Madre (BB-ULM) system is a hypersaline estuarine complex in South Texas that supports productive fisheries, coastal recreation, and regional economies. Understanding microbial water quality is essential for evaluating fecal contamination, protecting recreational water users, and informing coastal monitoring and management. This study aims to develop a microbial monitoring framework integrating fecal indicator bacteria (FIB), microbial source tracking (MST), and antimicrobial resistance (AMR). During the first seven months, monthly water sampling was conducted across twelve BB-ULM sites (N=83 water samples) to establish a baseline of microbial and molecular water quality conditions. Samples were tested for enterococci (100mL) using the IDEXX Enterolert method, while duplicate 100mL samples were concentrated on 0.45µm filters for DNA extraction and subsequent MST and AMR analyses. Preliminary results demonstrate spatial and temporal variability in enterococci concentrations, with occasional concentrations exceeding Texas Beach Watch's medium risk water quality threshold of 35-104 MPN/100mL. Sites BB5 and LM2 exhibited the highest enterococci concentrations and median values to date, identifying localized areas of elevated FIB within the BB-ULM system. Future work will complete the 12-month sampling period and initiate droplet digital PCR analyses targeting the human-associated fecal marker HF183 and class 1 integron-integrase gene *intI1*. Integrating these indicators will allow evaluation of relationships among general fecal contamination, human-associated contamination, and anthropogenic AMR potential. Continued monitoring and molecular analyses will strengthen the microbial baseline and provide information to support recreational water quality assessment, targeted monitoring, and science-based coastal management and conservation.

## FIRST REPORTS OF VECTOR-BORNE PATHOGENS IN URBAN FLEAS FROM DELAWARE

Amber Holley<sup>1</sup>, Giuseppe Troncoso Palacio<sup>1</sup>, Serhii Filatov<sup>1</sup>, Ashley Kennedy<sup>2</sup>, Maureen Laroche<sup>1</sup>

<sup>1</sup>University of Texas Medical Branch, Galveston, TX, United States. <sup>2</sup>Division of Fish and Wildlife, Newark, DE, United States

### Abstract

Fleas are vectors of many pathogens, including the causative agents of plague, murine typhus, and cat scratch disease. Accurate knowledge of the ecoepidemiology of fleas and their associated pathogens is crucial for informing clinical suspicion and diagnosis of flea-borne diseases, yet, in most countries, including the United States, flea surveillance is highly neglected. The State of Delaware recently confirmed the circulation of various flea species in urban environments; however, zoonotic pathogens have never been reported in fleas from that region. To investigate the circulation of bartonellae in fleas in Delaware, multiple flea species were collected from hosts in urban areas of Delaware and screened using molecular biology. Eight flea species were collected, including *Cediopsylla simplex*, *Ceratophyllus gallinae*, *Ctenocephalides felis*, *Doratomyssa blarinae*, *Epidemia wenmanni*, *Orchopeas howardi*, *Orchopeas leucopus*, and *Peromyscopsylla scotti*. *Bartonella* species were detected in 7.7% of *C. gallinae*, 5.3% of *C. felis*, 24.3% of *D. blarinae*, 31.1% of *O. howardi*, and 20% of *P. scotti*. Species-specific qPCR and sequencing revealed the presence of *B. henselae* and *B. silvicola* in *O. howardi* fleas respectively. Sequencing also revealed the circulation of two new *Bartonella* genotypes, proposed here as "Candidatus *Bartonella vinsonii* subsp. *breitschwerdtii*" and "Candidatus *Bartonella blarinini*". Our results are the first reports of *Bartonella* circulation in fleas from Delaware. These urban samples suggest a risk for transmission to humans and pets, which underscores the need for active flea surveillance programs across the U.S. to assess the risk of flea-borne pathogens that are neglected, such as bartonellae.

## Comparative Human Health Risk Assessment along the Texas Gulf Coast

Hye Seung (Lucy) Lee<sup>1</sup>, Valeria Ruvalcaba<sup>2</sup>, Nicole Powers<sup>3</sup>, Shaylynn Postma<sup>4</sup>, Lucas Gregory<sup>4</sup>, Anna Gitter<sup>2</sup>

<sup>1</sup>UTHealth Houston, Houston, United States. <sup>2</sup>UTHealth Houston, El Paso, United States. <sup>3</sup>Harte Research Institute, Texas A&M University Corpus-Christi, Corpus Christi, United States. <sup>4</sup>Texas Water Resources Institute, Texas A&M AgriLife, College Station, United States

### Abstract

Coastal recreational waters support public health, tourism, and local economies, but fecal contamination remains a persistent water quality concern, especially in Texas waters. Current monitoring practices rely on detecting fecal indicator bacteria (FIB) such as enterococci and *Escherichia coli* to assess recreational water safety. While widely used, FIB measurements cannot distinguish between human and non-human fecal sources, limiting their usefulness for identifying contamination sources and implementing targeted water quality management strategies. Microbial source tracking (MST) provides a promising approach for identifying source-specific fecal contamination and can inform human health risk assessments, which translate environmental data into public health risk. Water samples were collected from coastal recreational sites along the Texas coast, which included the communities of City-by-the-Sea, Corpus Christi, and Rockport. MST markers targeting human, gull, and canine fecal sources were quantified and utilized in a quantitative microbial risk assessment (QMRA) to estimate the risk of illness for recreators. Illness risks were compared with the U.S. Environmental Protection Agency recreational water illness risk benchmark of 32 illnesses per 1,000 recreators (0.032). Preliminary QMRA estimates indicate variability in illness risk across sampling locations and fecal contamination sources, with ingestion being a primary driver of health risks. Combining MST with QMRA provides a framework for linking fecal contamination sources with quantitative human health risk estimates. This approach may improve recreational water quality management by supporting targeted strategies that will focus on addressing pollution sources that not only impair water quality but are also of greatest concern for human health.

## **Investigating Historical Flow Condition Impact on Intermittent Stream Microbiomes in an Urbanized Watershed**

Jun Sheng Lee, Allison Veach

University of Texas at San Antonio, San Antonio, United States

### **Abstract**

Intermittent rivers make up ~ 30% of the total stream length in river networks globally. Intermittent river surface area is predicted to increase in the future because of climate change and water abstraction. Previous work in urban rivers in the San Antonio metropolitan area have indicated that intermittent reaches contain a high abundance of complex organic matter molecules with low bacterial/archaeal diversity in sediments. This project seeks to extend previous work in the San Antonio River Watershed by addressing at what point during dry-down (after a flow returning rainfall event) do bacterial/archaeal communities destabilize and if this divergence in diversity is driven by historical drought or sediment quality. After a ~2-inch rainfall event on June 12, 2025, water and sediment samples were collected over time (mid-June to mid-August) during dry-down after flow returned in San Antonio, Texas, from intermittent reaches. Perennial reaches were also temporally sampled as a comparison. Preliminary results indicate higher water-column nitrate concentrations ( $p = 0.03$ ) and orthophosphate ( $p = 0.05$ ) in perennial reaches compared to intermittent streams. This is likely attributed to the supply of nutrient-rich water from wastewater treatment plants and industrial runoff. Ongoing analyses will focus on estimating sediment dehydrogenase enzyme activity, a proxy for aerobic respiration, and water and sediment microbial communities (16S rRNA gene amplicons). Additional sampling campaigns will occur in 2027. These data are essential for understanding if intermittent river microbiome structure and function are linked to historical flow or due to alteration in carbon quality.

## Soil Fungal Diversity and Plant Pathogen Guilds in Urban Green Spaces Vary Spatially

Hannah Martinez, Allison Veach

University of Texas at San Antonio, San Antonio, United States

### Abstract

Urbanization alters soils via compaction and chemical pollution, which not only lowers soil quality but also reduces beneficial microbial consortia in soil profiles. Microbial communities drive nutrient cycling, enhance carbon storage, and provide important ecosystem services to urban communities. Yet, urbanization effects on fungal communities remain understudied in urban soils. This study examines fungal microbiomes across urban green spaces (parks, trails, and natural areas) in San Antonio, Texas, to identify how soil physicochemical properties and impervious surface cover structure soil fungal communities. Soil samples (n=39) were collected from 13 locations varying in vegetation and land management type and analyzed for soil physiochemistry (gravimetric water content (GWC), soil organic matter (SOM), total organic carbon (TOC), total nitrogen (TN), Mehlich-III phosphorus (P), and extractable cations (Ca, Mg, Na, and K)). Genomic DNA was extracted, and the ITS2 regions were PCR-amplified for Illumina MiSeq sequencing. All soil properties differed significantly among sites, tracking a land-use gradient in which greater impervious cover corresponded to lower soil organic matter and total organic carbon ( $p=0.001$ ). Fungal community composition also differed significantly among sites (PERMANOVA,  $R^2=0.414$ ,  $p=0.001$ ), with TN, Ca, SOM, and TOC most strongly correlated with fungal beta diversity. However, fungal alpha diversity metrics did not differ significantly among sites. ANCOVA models indicate that a subset of taxa in functional guilds responded significantly to site or soil covariates (Family Aspergillaceae, Phylum Ascomycota, the fungal guild Plant Pathogen). Overall, these findings highlight fungal communities as sensitive indicators of urban soil health in semi-arid cities.

## **Antimicrobial Strategies Graduate Student Posters**

## Leveraging Collateral Sensitivity to Protect Last-Line Antibiotics in *Acinetobacter baumannii*

Anna Evers, Rohan Siddanati, Nicholas Dillon

The University of Texas at Dallas, Richardson, United States

### Abstract

*Acinetobacter baumannii*, a critical-priority ESKAPE pathogen, rapidly acquires multi-drug resistance (MDR) leading to severe morbidity and mortality rates. MDR *A. baumannii* infections frequently necessitate colistin as a last-resort therapy; however, colistin resistance is now emerging. As we experience a dearth in antibiotic discovery, it is essential to counter the emergence of colistin resistance and protect the efficacy of last-line antibiotics. This underscores the need for therapies that not only successfully eliminate the infection, but also actively target the evolution of antibiotic resistance itself. One promising avenue is exploiting collateral sensitivity, where gaining resistance to one antibiotic increases susceptibility to another.

We generated several independent colistin-resistant lineages across multiple *A. baumannii* strains and found that the acquisition of colistin resistance consistently produced a significant decrease (up to 256-fold) in meropenem resistance; however, the reverse does not occur, revealing a robust, unidirectional collateral sensitivity relationship. While colistin-evolved strains retained their  $\beta$ -lactamase activity, they displayed a sharp increase in outer membrane permeability aligning with the decrease in meropenem resistance. Genomic analysis revealed that canonical colistin-resistance *lpxACD* mutations, although present, did not coincide with the onset of this phenomenon, indicating additional genetic changes may play a role.

These findings demonstrate that colistin resistance in *A. baumannii* generates a reproducible collateral sensitivity to meropenem involving increased membrane permeability. The ability of meropenem to disrupt colistin resistance evolution suggests that collateral sensitivity can be leveraged in novel treatments to suppress resistance trajectories and preserve efficacy of last resort antibiotics.

## Bicarbonate Suppresses the Phenotypic Expression of Azithromycin Resistance in *Pseudomonas aeruginosa*

Tuhina Maity, Nicholas Dillon

The University of Texas at Dallas, Richardson, United States

### Abstract

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CF transmembrane conductance regulator (CFTR) that impair chloride and bicarbonate transport across airway epithelia. This creates a dehydrated, acidic, and viscous airway environment that supports chronic infection by *Pseudomonas aeruginosa*. Azithromycin (AZM) is widely used in CF care, yet its resistance evolution is studied in standard bacteriological media. Here, we show that bicarbonate, a physiologically relevant ion, alters AZM resistance evolution.

*P. aeruginosa* P4 was resistant to AZM in bicarbonate-free CAMHB (MIC 256 µg/mL) but susceptible in RPMI (MIC 1 µg/mL). Adaptive laboratory evolution (ALE) produced high-level resistance in bicarbonate-free media, whereas resistance remained suppressed in bicarbonate-containing media. Removing bicarbonate from RPMI restored resistance evolution, while adding bicarbonate to CAMHB suppressed resistance, demonstrating that this phenotype is specifically associated with bicarbonate. To determine whether bicarbonate prevents resistance mutations or suppresses their phenotypic expression, evolved lineages were transferred between media, and AZM MICs were compared with parental MICs in transfer medium. Lineages evolved in bicarbonate-containing medium exhibited increased MICs after transfer to bicarbonate-free media, suggesting that bicarbonate suppresses the expression of resistance acquired during evolution.

Whole-genome sequencing identified *nfxB* mutations in bicarbonate-free evolved lineages. As other studies showed *nfxB* loss-of-function can induce MexCD-OprJ efflux pump expression, this pathway may contribute to AZM resistance in the absence of bicarbonate. Together, our findings suggest that bicarbonate constrains the phenotypic expression of resistance rather than preventing resistance-associated mutations, highlighting the importance of physiologically relevant environments in understanding antibiotic resistance evolution.

## Investigating a highly hydrophobic peptide produced by *Pseudomonas aeruginosa*

Sruthi Mudunuri<sup>1</sup>, Fabiha Zaheen Khan<sup>1</sup>, Aparna Uppuluri<sup>1</sup>, Shrinidhi Byregowda<sup>1</sup>, Kelli Palmer<sup>1</sup>, Ziqiang Guan<sup>2</sup>

<sup>1</sup>University of Texas at Dallas, Richardson, United States. <sup>2</sup>Duke University, Durham, United States

### Abstract

Bacteria compete with other bacteria for nutrients and space in their environments by altering food intake or by active killing. *Pseudomonas aeruginosa* is a gram-negative opportunistic pathogen that resides in competitive polymicrobial communities. It produces immense amounts of secondary metabolites, including antimicrobial compounds. In host physiological environments, these can help *P. aeruginosa* survive and infect the host, resulting in chronic, and sometimes, antibiotic resistant infections. The growing challenge of antibiotic resistance in treating diseases encourages us to understand novel compounds produced by bacteria that have potential antibacterial activity. Bacteriocins are protein products produced by some bacteria that have targeted bactericidal activity against competing bacteria while protecting themselves. Understanding and inhibiting these factors may slow infection progression or severity. In this study we identify a novel highly hydrophobic peptide produced by *P. aeruginosa*. We hypothesize that it has antibacterial activity against other bacteria. We identified that the peptide was produced when the cultures were grown in Lysogeny broth in aerobic shaking conditions with stationary phase growth but not in exponential or anaerobic growth conditions and confirmed its biosynthetic gene operon utilizing respective transposon mutants. Co-culture studies suggest that bacteriocin activity does not exist against *Staphylococcus aureus* and *Escherichia coli* under the conditions tested. Further, we will overexpress the peptide under an IPTG-inducible promoter and test its antibacterial activity against other bacteria. A deep understanding of these antibacterial mechanisms is crucial for the design of therapeutic strategies and vaccines against multi-resistant pathogens.

## EXPLORING THE ANTI-STAPHYLOCOCCUS AUREUS ACTIVITY OF *DOLOSIGRANULUM PIGRUM*

Abiola Obawemimo<sup>1</sup>, Allison Judge<sup>1,2</sup>, Chad Johnston<sup>1,2</sup>, Katherine Lemon<sup>1,3,4</sup>

<sup>1</sup>Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, United States. <sup>2</sup>Department of Biochemistry & Molecular Pharmacology, Baylor College of Medicine, Houston, United States. <sup>3</sup>Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, United States. <sup>4</sup>Division of Infectious Diseases, Texas Children's Hospital, Department of Pediatrics Baylor College of Medicine, Houston, United States

### Abstract

*Staphylococcus aureus* is the second leading bacterial cause of death globally. Approximately 30% of people are nasally colonized, which increases infection risk. ~80% of infection strains match the person's nasal strain. No effective human vaccine exists. However, *S. aureus* colonization negatively correlates with some nasal microbionts, including *Dolosigranulum pigrum*.

About 60% of our human nasal *D. pigrum* isolates inhibited *S. aureus* on agar medium. AntiSMASH analysis predicted strain-variable biosynthetic gene clusters (BGCs) that might contribute to this antagonism. *D. pigrum* grows best in or on semi-solid medium. With *D. pigrum* grown embedded in agarose medium, the activity remained in the agarose after extraction with multiple different solvents, which is unusual.

However, when we grew *D. pigrum* atop a 0.2 µm polycarbonate membrane placed on a BHI 10% polyacrylamide gel (PAG), centrifugation of the cell-free conditioned PAG (CFCPAG) resulted in an aqueous extract with inhibitory activity. Ultrafiltration using a 100-kDa cutoff showed activity exclusively in the >100 kDa fraction (n > 3), suggesting the activity is either a high-molecular-weight (HMW) compound, a HMW-associated compound, or a highly cationic small compound. The >100 kDa extract was inactivated by proteinase K, trypsin, amyloglucosidase, and heat ≥ 45°C, suggesting a highly cationic peptide, glycoprotein, HMW or HMW-associated compound. Interestingly, activity from *D. pigrum*-embedded agarose was also proteinase K-sensitive but more thermostable (inactivated at 75°C), suggesting two potentially distinct compounds.

Future directions include purification and characterization of the *D. pigrum* anti-*S.aureus* compound(s) to explore potential therapeutic use in *S. aureus* nasal decolonization

## Patterns of Collateral Sensitivity and Collateral Resistance Across Mechanistically Distinct Antibiotics in *Pseudomonas aeruginosa*

BARUTI OREDY

Texas Tech University, Lubbock, United States

### Abstract

The global rise in antibiotic-resistant bacterial diseases poses a significant threat to global health. Multidrug-resistant bacteria diminish the efficacy of common antibiotics against widespread bacterial infections. In our research, we investigated antibiotic resistance in *Pseudomonas aeruginosa*, particularly among cystic fibrosis (CF) and associated isolates. We sought to explore Collateral sensitivity (CS) and collateral resistance (CR) to shape how antibiotic resistance evolves and persists across drug classes. We looked for patterns of CS and CR across a diverse collection of CF-associated *P. aeruginosa* isolates. We tested antibiotic susceptibility by using minimum inhibitory concentrations (MICs) that we measured for MDR CF isolates, mucoid CF isolates, and laboratory reference strains against a panel of 14 antibiotics. We used Pearson correlation coefficient (r) analysis to map out antibiotic responses. The results revealed substantial collateral resistance within antibiotic classes and among drugs sharing uptake routes or resistance mechanisms. Importantly, we observed that multiple antibiotic pairs from distinct mechanistic classes exhibited significant collateral sensitivity. We identified negative correlations between novobiocin (DNA gyrase inhibitor) and aztreonam (PBP3-targeting  $\beta$ -lactam), as well as between tobramycin (aminoglycoside) and ampicillin ( $\beta$ -lactam). These strong negative correlations suggest that resistance to one antibiotic may be associated with increased susceptibility to another. Interestingly, some antibiotic pairs with distinct molecular targets demonstrated collateral resistance despite mechanistic differences. We also observed partial CR between fluoroquinolones and carbapenems. Our study provides a mechanistic basis for selecting candidate antibiotic combinations that exploit collateral sensitivity and limit resistance development.

## Systematic Discovery and Experimental Validation of Collateral Sensitivity to Antibiotics in *Pseudomonas aeruginosa*

Md Siddiqur Rahman, Baruti Oredy, Angelina Marranzini, Emmanuella Yeboah Asiamah, Hannaneh Ghavanloughajar, Catherine Wakeman

Texas Tech University, Lubbock, United States

### Abstract

Antibiotic resistance in *Pseudomonas aeruginosa* poses a serious global health threat, particularly in the treatment of chronic or hospital-acquired infections. However, new antibiotics are not being developed quickly enough to keep pace with the resistance evolution in this pathogen. Therefore, therapeutic strategies that can suppress this resistance emergence and extend the efficacy of existing drugs are urgently needed. One such strategy is the identification of collateral sensitivity (CS), wherein resistance development to one antibiotic increases susceptibility to another. Though CS dynamics can guide treatment design to impede resistance development, this has not previously been systematically explored in clinical *P. aeruginosa* isolates. In our study, we determined minimum inhibitory concentrations (MICs) for 23 antibiotics across a panel of 33 clinical isolates, and used correlation analysis of these profiles to screen for candidate CS pairs. We then combined this screening approach with adaptive laboratory evolution in reference strain PA14 and successfully validated 15 CS pairs. We also developed a relationship network among these validated CS pairs to visualize the CS pattern across independently evolved lineages. In future work, we plan to pinpoint the genetic basis of these trade-offs through whole-genome sequencing and to test whether the validated CS pairs can be deployed as sequential or combination regimens to suppress resistance *in vivo*. Overall, these findings provide a clinically grounded framework for identifying exploitable CS relationships in *P. aeruginosa* and offer a promising direction for designing evolution-informed antibiotic treatment strategies.

## Disruption of Polymicrobial Synergism Through Combinatorial Treatment

Jace Salgado<sup>1</sup>, Caroline Laughlin-Black<sup>2</sup>, Catherine Wakeman<sup>1</sup>

<sup>1</sup>Texas Tech University, Lubbock, United States. <sup>2</sup>University of Texas at Dallas, Dallas, United States

### Abstract

Antimicrobial resistance (AMR) is a major global public health threat that increasingly limits the effectiveness of conventional antibiotic therapy. This challenge is further complicated in chronic wound infections, where polymicrobial communities can promote antibiotic recalcitrance and cross-species protection. Previous work in our lab demonstrated that the presence of a polymicrobial community can protect *Enterococcus faecalis* from cephalexin treatment. We have shown that this protection can be mediated by both *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, both of which are frequently found co-infecting sites of chronic *E. faecalis* infection. This protection is mediated by  $\beta$ -lactamase production from the Gram-negative pathogens. The goal of this study is to disrupt this polymicrobial synergism and eradicate the entire community by targeting these keystone Gram-negative pathogens that are protecting *E. faecalis*. Targeted disruption will be achieved through combinatorial treatment with either colistin or bacteriophages. Promising bacteriophages for this work are being isolated from wastewater treatment plants. We hypothesize that targeted eradication of keystone Gram-negatives could disrupt  $\beta$ -lactamase-mediated protection of *E. faecalis* and improve  $\beta$ -lactam activity within a polymicrobial wound community. Experiments will be performed in standard susceptibility medium, Lubbock chronic wound medium, and artificial CF sputum medium to compare treatment effects under conventional versus chronic infection-relevant conditions. This combinatorial approach may represent a novel treatment strategy that preserves the efficacy of existing antibiotics, which is imperative to combating the growing threat of AMR.

## Growth temperature impacts antibiotic efficacy in *Pseudomonas aeruginosa*.

Adolph Salinas<sup>1</sup>, Catherine Wakeman<sup>1</sup>, Alex Leuke<sup>1</sup>, Deborah Bajomo<sup>2</sup>

<sup>1</sup>Texas Tech University, LUBBOCK, United States. <sup>2</sup>Columbia University, New York, United States

### Abstract

*Pseudomonas aeruginosa* is a ubiquitous Gram-negative rod-shaped bacillus bacteria often encountered in soil, bodies of water, as well as the mammalian host. Most commonly associated with hospital acquired infections, this bacterium can become an opportunistic pathogen causing potentially life-threatening infections in the elderly, people with Cystic Fibrosis, and those with compromised immune systems. *P. aeruginosa* is a highly virulent member of the ESKAPE pathogens, a group of six bacteria that are multidrug resistant, making them increasingly difficult to treat. An added difficulty can be attributed to the fact that microbial physiology may change within different growth conditions, leading to altered antibiotic efficacy relative to the standard clinical laboratory antimicrobial susceptibility testing results. One difference that occurs between the diverse growth sites of *P. aeruginosa* is the shift in temperature. *P. aeruginosa* is exposed to 23 °C when growing on abiotic surfaces of the clinic. However, within the host niche, the temperatures may range from ambient in the anterior nares, to 30-33 °C within the wound bed, to core human body temperature (37 °C) in deeper infections such as the lung. We have determined the minimal inhibitory concentrations of a variety of antibiotics at this temperature range and have identified numerous shifts of potential clinical importance. Future research is required to determine the clinical significance of these susceptibility shifts, especially since other growth-related factors such as biofilm formation should also be considered.

## Lipopolysaccharide Remodeling Confers Resistance to Bam Complex Inhibitors

Teresa Sullivan<sup>1</sup>, Anna Kononova<sup>2</sup>

<sup>1</sup>The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, Houston, United States. <sup>2</sup>The University of Texas Health Science Center at Houston, Houston, United States

### Abstract

Antimicrobial resistance (AMR) is a major global health problem. Many of the serious threats are gram-negative bacteria, which have intrinsic resistance to many antimicrobials due to the impermeable outer membrane. To combat this, inhibitors have been developed that target BamA, an essential component of the  $\beta$ -barrel assembly machinery (Bam) complex that assembles outer membrane proteins (OMPs). However, resistance to Bam complex inhibitors is largely unstudied. Therefore, an understanding of resistance mechanisms is essential to proactively address the emergence of resistance to such inhibitors and develop anti-resistance strategies. Our lab isolated spontaneous MRL-494 resistant mutations in *E. coli* that directly or indirectly modify the lipopolysaccharide (LPS) structure. Using genetic and biochemical analyses, I have investigated the molecular mechanism and the clinical implications of this resistance. Preliminary investigation revealed that this resistance is dependent on LPS modifications and downstream of Bam complex inhibition. Consistent with these results, I've shown that this resistance is non-specific to MRL-494. It applies more broadly to Bam complex inhibiting conditions and promotes resistance to another BamA inhibitor, darobactin. I've shown that upon Bam inhibition, LPS levels decrease in the susceptible wild-type strain, while they are maintained in the resistant mutant. I hypothesize that this stabilization of LPS retains the impermeable and rigid nature of the outer membrane, promoting continued cell survival. Based on my results, I have found that compensatory remodeling of outer membrane lipid components can improve cell survival during Bam complex inhibition and that LPS structure plays a significant role in Bam complex inhibitor activity.

## Identifying Genetic Determinants of R Pyocin Sensitivity in *Pseudomonas aeruginosa*

Meggie Wang, Yanhan Deng, Chandni Chopra, Natasha Kirienko  
Rice University, Houston, United States

### Abstract

*Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogen, is a highly antibiotic-resistant bacteria that causes a variety of acute and chronic infections in immunocompromised patients and particularly in people with cystic fibrosis. *P. aeruginosa* infections are predominantly caused by a small number of sequence types (STs) such as ST235, ST111, and ST253. Previous research in the Kirienko Lab linked the dominance of these STs to the production of R pyocins, phage tail-like bactericidal protein complexes that target non-kin strains of *P. aeruginosa*. R pyocins bind to the core residues of lipopolysaccharide (LPS), and kill target cells by puncturing the outer membrane, leading to ion leakage. This phenomenon is well described in laboratory *P. aeruginosa* strains, but studies in clinical strains, or understanding of genetic determinants of R pyocin sensitivity are limited.

To uncover the patterns of R pyocin sensitivity, we investigated clinical isolate genomes using bioinformatics tools to identify mutations in genes that are unique to sensitive strains. This analysis generated an exhaustive list of sensitivity-associated genes which were screened using a commercially-available transposon mutant library. We identified several genes that affected R pyocin sensitivity, including known LPS-synthesis genes *wbpL* and *wbpM*, and the novel O-antigen ligase, *waaL*. Laboratory-generated *waaL* deletion strains in clinical and laboratory strain backgrounds showed increased sensitivity to R pyocins. Further investigation into the genetic patterns of R pyocin susceptibility in *waaL* deletion mutants may reveal other factors in the dynamics of *P. aeruginosa* clinical dominance and further the development of R pyocins as therapeutics against multidrug-resistant infections.

## Analysis of Fidaxomicin Resistance Development in *Clostridioides difficile*, Using Experimental Evolutions

Jiselle Zavala, Chetna Dureja, Julian Hurdle

Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology,  
Department of Translational Medical Sciences, Texas A&M Health Science Center, Houston, United States

### Abstract

In 2011, the FDA approved fidaxomicin (FDX) for the treatment of *Clostridioides difficile* infection (CDI), and in 2021, the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) recommended FDX as the first-line treatment for CDI. Since its introduction, FDX resistance has been documented in clinical isolates; resistance is frequently associated with mutations within the fidaxomicin-binding pocket of the target enzyme, RNA polymerase (RNAP), including mutations in the *rpoB* and *rpoC* subunits. However, there remains a gap in our understanding of how FDX resistance emerges and the mechanisms underlying resistance in patients. In this study, we propose the use of serial experimental evolution under batch culture conditions to select FDX-resistant mutants. This presentation will discuss the results emerging from this evolutionary study, which will provide insight into resistance mechanisms associated with reduced fidaxomicin susceptibility and evaluate the stress response resulting from the disruption of RNAP function in *C.difficile*. By better understanding the development of FDX resistance, this work will help inform clinical strategies for treating CDI and improve treatment outcomes for patients.

## Genomics Graduate Student Posters

## Developmental Programming of Gut Microbial Metabolic Niches

Morenikeji Adedayo, Aaron Wright

Baylor University, Waco, United States

### Abstract

Early childhood is a critical period for microbial colonization and metabolic niche establishment, and immune development, yet the timing and functional maturation of specific microbial pathways remain poorly defined. While the taxonomic compositional patterns of the gut microbiome during this critical window are well characterized, it remains unclear when specific microbial functions, particularly those involved in carbohydrate utilization and xenobiotic metabolism, emerge and stabilize. Deciphering the developmental trajectory of the gut microbiome's metabolic capacity will enhance our ability to link early-life microbial development to downstream metabolic and immune outcomes.

To address this, we developed a structure-informed metagenomics framework to resolve microbial enzyme niches across early-life development. This approach integrates longitudinal metagenomic datasets with curated reference enzyme families and applies reciprocal homology searches, domain-based filtering, and clustering to identify high-confidence functional gene sets beyond conventional annotation methods. By incorporating structural and motif-level constraints, this pipeline improves specificity in assigning enzyme function and enables reconstruction of enzyme-level abundance profiles across developmental time.

We are applying this framework to longitudinal infant microbiome datasets with rich metadata on diet, early-life exposures, and perinatal factors. Preliminary implementation demonstrates robust recovery of target enzyme families and reveals heterogeneous, time-dependent patterns of enzyme abundance across samples, consistent with a model of stage-specific functional maturation.

Together, this work establishes a function-centric view of microbiome development, providing a foundation for identifying critical windows of functional maturation and potential targets for early-life intervention.

## Spilling the guts on *Candida albicans*: Genomic and phenotypic diversity of isolates derived from the human digestive tract

Madhvi Bhakta<sup>1,2</sup>, Elena Lindemann-Perez<sup>2</sup>, Max W. Adelman<sup>3</sup>, Rodrigo De Paula Baptista<sup>3</sup>, Michael C. Lorenz<sup>1,2</sup>, J Christian Perez<sup>1,2</sup>

<sup>1</sup>The University of Texas MD Anderson Cancer Center UTHHealth Graduate School of Biomedical Sciences, Houston, United States. <sup>2</sup>Department of Microbiology and Molecular Genetics, UTHHealth Houston McGovern Medical School, Houston, United States. <sup>3</sup>Center for Infectious Diseases, Houston Methodist Research Institute, Houston, United States

### Abstract

*Candida albicans* is a ubiquitous fungal pathobiont residing in the human digestive tract. It is also the most common cause of life-threatening disseminated fungal infections in developed countries. The genetic and phenotypic diversity of *C. albicans* in the human gut, its primary natural habitat, remains underexplored because most research has emphasized isolates derived from active infection sites. To address this knowledge gap, we are characterizing >100 *C. albicans* isolates of human intestine origin collected in the Texas Medical Center. We used short Illumina and long Nanopore reads to sequence and assemble the genomes of all these isolates. Phylogenetic reconstruction based on orthologous single-copy genes indicates that this collection of gut *C. albicans* isolates represents all major branches of the species' phylogeny, intermingling with isolates derived from other body sites and collected in multiple other countries. Consistent with this wide genomic diversity, phenotypic profiling of the isolates is revealing extensive phenotypic variance as well. For example, while most isolates showed baseline levels of susceptibility/resistance to the common antifungal fluconazole in Etest strips, we identified independent isolates that displayed resistance, which seems linked to a previously undescribed chromosomal aneuploidy. We also observed substantial variation in host cell damage potential across isolates and found that this trait positively correlated with the isolates' propensity to filament under certain *in vitro* and *in vivo* conditions. Our findings are starting to reveal the broad genetic and phenotypic diversity of this fungal species in the human gut.

## Using GWAS to Mine for Compensatory Mutations in Cystic Fibrosis Adapted *Pseudomonas aeruginosa*

John Camp, Nicholas Dillon

University of Texas at Dallas, Richardson, United States

### Abstract

*Pseudomonas aeruginosa* is an ESKAPE pathogen that commonly infects Cystic Fibrosis (CF) patients through the compromised lung environment.

An ongoing problem for treatment of CF *P. aeruginosa* is its ability to maintain its fitness despite antibiotic treatment and conditions within the host, despite adaptive alleles being costly. One way *P. aeruginosa* attempts to solve this problem is through the formation of compensatory mutations, which are epistatic to the negative fitness consequences of adaptive alleles. I hypothesize that *P. aeruginosa* develops multiple compensatory mutations in response to antibiotic pressure and CF conditions.

To test this hypothesis; two parallel GWAS, one on CF and one non-CF *P. aeruginosa* datasets, will be utilized. The studies will be followed by comparative genomics between the two GWAS. Current results include a GWAS done on non-CF strains treated with Levofloxacin revealed a strong association with well-known fluoroquinolone mutations *gyrA* and *parC*. The GWAS also revealed multiple candidate compensatory mutations. Future work will focus on the role of identified compensatory mutations in shaping *P. aeruginosa* adaptation to the CF lung.

This work will establish a pipeline for rapidly identifying compensatory mutations and help elucidate how *P. aeruginosa* maintains fitness in the CF lung.

## Local Chicken, Global Concern: Resistance Genes and Virulence Traits in *Salmonella enterica* Isolates

Samuel Cannon, Madison Schultz, Marcos Montelongo, Layla Behrens, Aaron Lynne, Todd Primm, Anand Karki

Sam Houston State University, Huntsville, United States

### Abstract

Consumption of contaminated retail poultry products is the primary route of transmission of *Salmonella enterica*. In our recent study, 31% of retail chicken liver samples collected from grocery stores in Walker County, TX were found to be contaminated with diverse serovars of *Salmonella enterica*. In this study, we performed comparative genomic analysis of twelve selected *Salmonella enterica* subsp. *enterica* strains using long-read whole genome sequencing followed by genome annotation, including O/H antigen identification, multi-locus sequence-typing, and whole genome phylogenetic analysis. All collected isolates except one contained the classically described virulence plasmid with five isolates containing two or more plasmids of size varying from 3.6–284 kb. The phylogenetic analysis comported with MLST and subsequently serotype with note that several isolates in MLST 32 being non-serotypeable due to lack of O antigen presentation. The other identified MLST's were MLST 11 and 152 which align with serotypes Enteritidis and Kentucky respectively.

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 concern in retail chicken products. Drug resistance data also indicates frequent presence of MDR *Salmonella* in commercial chicken products.

## Navigating the protein dark space of lipoprotein N-acyltransferase in *Bacteroides* (Lnb)

Elizabeth Jeffery, Krista Armbruster, Nicole Koropatkin

University of Michigan, Ann Arbor, United States

### Abstract

Lipoproteins are a class of membrane protein involved in essential processes, including nutrient acquisition and immunomodulation. Lipoprotein N-acyltransferase in *Bacteroides* (Lnb), which contains the catalytic domain DUF4105, is responsible for catalyzing lipoprotein triacylation in *Bacteroides*, a genus that lacks canonical apolipoprotein N-acyltransferase (Lnt). However, the functional and evolutionary landscape of Lnb remains largely uncharacterized. In this work, we aimed to navigate the protein dark space to characterize the presence of Lnb across bacterial taxa and explore sequence and structure conservation. To achieve this aim, we constructed a novel profile hidden Markov model (pHMM) of DUF4105 using 995 protein sequences from the UniRef90 database that met our inclusion thresholds. Our pHMM identified 1,581 Lnb homologs, which we then clustered and aligned to generate a phylogenetic tree. Proteins originating from various *Bacteroides* species as well as more distantly related taxa were then manually examined using their UniProt accession IDs and AlphaFold-predicted structures. Preliminary results demonstrate that the DUF4105 sequence and structure are well-conserved among *Bacteroides* species. However, Lnb-like proteins within some phylogenetic clades were found to have a multidomain architecture containing the conserved DUF4105 domain, an uncharacterized  $\beta$ -barrel (DUF7840) domain, a predicted  $\alpha$ -helical bundle (DUF7842), and a predicted  $\alpha$ -helical array (DUF7843). We hypothesize that these DUF domains are involved in membrane anchoring. Further research is warranted to confirm the biochemical function of Lnb-like proteins across taxa. To the best of our knowledge, this is the first systematic investigation into the evolutionary relationships and domain architecture diversity of Lnb and Lnb-like proteins.

## Polymicrobial catheter-associated urinary tract infections persist and adapt over time

Cora Kosnik<sup>1</sup>, Jesus Duran Ramirez<sup>1</sup>, Jennifer N Walker<sup>1,2</sup>

<sup>1</sup>McGovern Medical School, Houston, TX 77030, United States. <sup>2</sup>School of Public Health, University of Texas Health Science Center at Houston, Houston, TX 77030, United States

### Abstract

Urinary catheters are often employed for the treatment of people who are hospitalized or critically ill, have urinary retention or neurogenic bladder dysfunction, or are elderly, making them the most frequently placed medical device. Catheter-associated urinary tract infections (CAUTIs) are a common complication of urinary catheterization, with >1 million cases annually in the US. CAUTIs are associated with increased morbidity and mortality, and as the US population ages, their costly impact on healthcare systems will continue to rise. Complicating their treatment is the high occurrence of antimicrobial resistance (AMR) among uropathogens, as well as the development of asymptomatic bacteriuria (ASB) – reservoirs of bacteria in the urine – which allows them to persist despite catheter changes and antibiotic treatment. Long term urinary catheters (LTUCs; >28-day dwell time) are also typically polymicrobial. Interspecies interactions during ASB and CAUTI can enhance uropathogenesis leading to increased mortality and development of severe disease such as pyelonephritis, kidney damage, and secondary bacteremia. Our preliminary data from longitudinally collected LTUC clinical isolates suggests that *Staphylococcus aureus* and *Enterococcus faecalis* frequently and persistently co-colonize, occurring at a rate of 65% in the study cohort. Strains that persist in the host undergo genomic adaption and accumulate changes in genes with implicated functions in fitness, AMR, and pathogenesis. By analyzing genomic changes over time, we seek to investigate genomic adaptations and potential co-interactions that aid in polymicrobial ASB persistence. We also performed coculturing experiments with *S. aureus* and *E. faecalis* and measured phenotypic outcomes among longitudinal strains to characterize these interactions.

## Unexpected positive selection on a malonate utilization gene revealed by genomic analysis across strains of *P. aeruginosa*

Alex Luecke, Catherine Wakeman, Amanda Brown

Texas Tech University, Lubbock, United States

### Abstract

*Pseudomonas aeruginosa* is an opportunistic pathogen known to establish itself in a wide variety of ecological niches, such as chronic and acute infections, industrial and natural water systems, and soil. As *P. aeruginosa* transitions from niche to niche, its available nutrients change, and therefore its carbon utilization pathways *P. aeruginosa* should change in response. For example, a previous study showed upregulation of malonate utilization genes, such as *mdcA*, when *P. aeruginosa* was grown in trauma patient blood, which was associated with an increased risk of sepsis. Here, we aimed to investigate how deeply conserved *mdcA* is relative to other carbon utilization genes across currently available *P. aeruginosa* sequences on NCBI. To do so, we built a phylogenetic tree from a core-gene alignment in Roary and then used Hyphy FEL to estimate the global dN/dS of various carbon utilization genes. Our study showed that the selected carbon utilization genes, regardless of isolation source, were under negative selection. Interestingly, *mdcA* had one site under strong positive selection that we believe may help modulate the efficacy of the malonate decarboxylase complex (*mdcA-mdcC-mdcD-mdcE*); however, further experiments will be needed to confirm this functional advantage. By understanding the selective pressures on carbon utilization genes such as *mdcA*, we may better characterize the niche-specific nutritional shifts of *P. aeruginosa* isolates in clinical infections and identify targets to minimize their growth.

## Tracing the Evolutionary Origins of Nitrogenase-Like Enzymes

Brittney Moser, Ulises Rodriguez-Cruz, Morgan Sobol

Texas State University, San Marcos, United States

### Abstract

Enzymes, central to the reactions sustaining life, often evolve through gene duplication and functional divergence. Yet the pressures driving their divergence, and the molecular and ecological consequences that follow, remain unclear for most enzyme families. One such family is the nitrogenase-like superfamily, found only in prokaryotes, whose members catalyze reactions spanning nitrogen fixation, chlorophyll and coenzyme F430 biosynthesis, and anaerobic sulfur respiration. Two of these homologs, methylthioalkane reductase (Mar) and isethionate reductase (Isr), were recently characterized, owing to repeated database misannotation and leaving their genomic distribution unexamined. Consequently, this family's evolutionary history remains distorted. Here, we reconstructed phylogenetic trees and analyzed the genomic context in which these enzymes reside to resolve their history. Hidden Markov Model (HMM) profiles were built for each subunit of all homologs, screened against a genome database, and iteratively refined. HMM assignments were additionally checked against a clustering-purity metric. Nitrogenase (Nif) proved most widespread, consistent with its role in nitrogen cycling. For Mar and Isr, independent analyses repeatedly placed the same organisms at an unresolved boundary, consistent with a more recent divergence than from any other family member. Co-occurrence analysis confirmed that the alternative nitrogenases almost never occur without Nif, while Mar and Isr each associate with genomes carrying canonical nitrogenases. This work offers the first genome-scale reconstruction of the superfamily's history and a broader case study in enzyme evolution. Future work will characterize the microorganisms hosting these enzymes to clarify their ecological roles and the selective pressures driving the evolution of this enzyme family.

## **Virology Graduate Student Posters**

## **Global Lipidomic Analysis of Lytic KSHV Infection: The Lipid Chaperone FABP4 Supports Maximal Infectious Virion Production**

Eranda Berisha, Erica Lee Sanchez

The University of Texas at Dallas, Richardson, United States

### **Abstract**

Kaposi's Sarcoma Herpesvirus (KSHV), an enveloped double-stranded DNA virus, is the etiological agent of Kaposi's Sarcoma (KS), an endothelial cell-based tumor. KSHV is a leading cause of infection-related cancers in sub-Saharan Africa and immunocompromised individuals worldwide. Therefore, it is vital to identify the underlying mechanisms of viral infection and transmission to effectively identify specific therapeutic strategies and combat the disease. We have demonstrated that KSHV rewires the host cell lipidome during lytic infection. Bulk lipidomic analysis shows significant changes in the abundance of neutral lipids and phospholipids during lytic infection. We further investigated fatty acid binding proteins (FABPs) to understand the underlying mechanisms that support KSHV pathogenesis. Using the doxycycline-inducible iSLK.BAC16 cell line, we find that FABP genes are differentially regulated by lytic KSHV infection compared to latent infection. We report that FABP4 is significantly upregulated during lytic infection. FABP4 knockdown using siRNA or inhibition of the FABP4 protein via treatment with a competitive inhibitor during lytic infection significantly reduces extracellular viral titers, indicating that FABP4 supports maximal infectious virion production. Our data highlights the role of FABP4 as a host target that facilitates KSHV infection and pathogenesis.

## Investigating mRNA Condensation Escape from Stress Granules During Chikungunya Infection

Maxine Cañelas, Jared Bard

Texas A&M University, TX, United States

### Abstract

Stress granules (SGs) are biomolecular condensates composed of proteins and stalled mRNAs that form in response to cellular stress, such as in Chikungunya viral infection. SGs are hypothesized to form as an antiviral response by sequestering transcripts and inducing global translational repression that inhibits both host and viral gene expression. However, studies in yeast have shown that a subset of nascent mRNAs escape condensation into SGs following heat shock and are translationally upregulated. Whether this phenomenon occurs in mammalian cells and how different conditions influence stress-specific transcript condensation escape remains unknown. To address this gap, we will use APEX proximity labeling of mRNAs associated with a key SG nucleator protein, G3BP1, combined with 4sU metabolic labeling to discriminate between nascent and pre-existing mRNAs within SGs. We predict that transcripts promoting antiviral response, such as interferon-stimulated genes (ISGs), will escape condensation to promote viral clearance by the host cell.

## LANA-dependent interactions of DAXX and BRD4 regulate KSHV lytic replication

Maria del Carmen Chacon Castro<sup>1</sup>, Erica Lee Sanchez<sup>1</sup>, Robyn M. Kaake<sup>2</sup>, Rithika Adavikolanu<sup>2</sup>

<sup>1</sup>The University of Texas at Dallas, Richardson, United States. <sup>2</sup>UCSF, San Francisco, United States

### Abstract

Kaposi's Sarcoma-associated Herpesvirus (KSHV) is an oncogenic gammaherpesvirus responsible for Kaposi's Sarcoma (KS). To establish lifelong infection, KSHV manipulates host cellular pathways, gene expression programs, and metabolic processes while switching between latent and lytic phases. During latency, the viral Latency-Associated Nuclear Antigen (LANA) is required for viral episome maintenance and regulation of viral persistence. Although LANA is known to interact with numerous host proteins, the impact of these interactions on host protein networks and their role in controlling latency and reactivation is not fully understood. Using immunoprecipitation-mass spectrometry (IP-MS), we identified Death Domain-Associated Protein (DAXX) as a major host protein interacting with LANA. We also identified a LANA-dependent recruitment of the chromatin regulator Bromodomain-Containing Protein 4 (BRD4) to DAXX, revealing a previously unrecognized alteration of the DAXX protein interaction network during KSHV infection. Functional studies using siRNA-mediated depletion of these host factors in iSLK.BAC16 cells, followed by lytic reactivation, showed that loss of DAXX or BRD4 significantly increased the expression of KSHV lytic genes, including ORF45, ORF59, ORF26, and K8.1. This increase was accompanied by enhanced viral genome replication and production of infectious virions. Similarly, pharmacological inhibition of BRD4 with JQ1 produced effects comparable to BRD4 knockdown. Collectively, these findings identify DAXX and BRD4 as key host regulators of latency, highlighting potential therapeutic targets for KSHV-associated malignancies.

**To study the intrinsically disordered regions of DEAD-box helicases and their involvement in protein-protein interactions and antiviral functions using a systematic variant library and imaging-based colocalization assays.**

Pragati Dhingra

Texas A&M University, College Station, United States

**Abstract**

DEAD-box helicases are a family of ATP-dependent RNA helicases that play critical roles in RNA metabolism, stress responses, and antiviral defense. DEAD-box helicases including DDX41, DDX56, and DDX60 have established antiviral roles, while others such as DDX3X exhibit proviral functions and DDX5 and DDX17 display context-dependent dual roles, yet the molecular basis for these functional differences remains unclear. While their conserved helicase core domains are well characterized, the intrinsically disordered regions (IDRs) found in their variable N- and C-termini remain poorly understood, particularly with respect to their roles in mediating protein-protein interactions. This research investigates the chemical and structural properties of DDX IDRs and their functional significance in antiviral responses.

Using a sequential bioinformatics and experimental approach, the chemical properties of DDX IDRs are analyzed to identify evolutionarily conserved features in DEAD-box helicases known for their antiviral functions, comparing these features to DDXs with proviral or dual functions. In future experiments, these chemical features will then be functionally validated through systematic mutations in select DDXs. Variant libraries will be screened to assess the functional consequences of IDR mutations on pro- or antiviral activity.

This research is expected to build an understanding of how specific chemical properties encoded within disordered protein regions drive biologically relevant interactions, with potential applications in the therapeutic targeting of viral proteins.

## **GNMT as a candidate diagnostic and prognostic marker for HCV-mono- and HIV/HCV-co-infection-mediated hepatocellular carcinoma.**

Tristan Flores<sup>1</sup>, Fahed Aljiboury<sup>2</sup>, Pankaj Chaudhary<sup>1</sup>, Park In-Woo<sup>1</sup>

<sup>1</sup>UNT Health, Fort Worth, United States. <sup>2</sup>UT Austin, Austin, United States

### **Abstract**

We investigated molecular mechanisms of HIV-1-mediated acceleration of liver disease progression toward HCC in HCV-infected patients and sought to identify potential biomarkers critical for accurate diagnosis and prognosis of clinical stages of HCC. Our transcriptome array indicated that Glycine-N-Methyltransferase (*gnmt*) expression was significantly suppressed in a liver-disease stage-specific manner in the mono- and co-infected patients, indicating that the level of expression is clearly liver disease stage- and mono-/co-infection specific. TCGA-LIHC analysis indicated low *gnmt* expression was significantly associated with poor survival in HCC patients, corroborating the significance of *gnmt* in HCC. Moreover, expression of GNMT significantly reduced expression of the subgenomic replicon of the JFH1 strain of HCV. In contrast, HIV-1 Nef, a key pathogenic molecule of HIV, substantially enhanced replicon expression while regulating the amount of GNMT. Co-expression of HIV-1 *nef* and *gnmt* in the replicon cells showed that the positive impact of *nef* on HCV replication outweighed *gnmt*-triggered negative effects, indicating that HIV-1 Nef and GNMT are the cardinal regulators for the co-infection-mediated expedition of HCC progression. Further, Nef and GNMT interplayed for regulation of HCV replicability. Our proteomic analysis further showed that several cellular proteins are involved in Nef- and GNMT-mediated regulation of HCV replication. These findings will provide pivotal information to elucidate the molecular mechanisms underlying the acceleration of HCC progression in HIV-1 co-infection. Likewise, identification of GNMT/Nef-associated differentially expressed cellular genes could be exploited as biomarkers and therapeutics in HIV-1/HCV co-infected patients.

## **Defining Endothelial Cell and Sensory Neuron Communication Networks During Kaposi's Sarcoma-Associated Herpesvirus Infection.**

Caylah Griffin, Anjali Venna, Michael Burton, Erica Sanchez

University of Texas at Dallas, Richardson, United States

### **Abstract**

Kaposi's Sarcoma (KS) is the most common HIV-associated cancer caused by Kaposi's Sarcoma Associated Herpesvirus (KSHV). KS originates in the dermis and the lesion consists of heterogeneous cell populations recruited by KSHV-infected endothelial cells (ECs) through cytokine and interferon signaling, contributing to angiogenesis and plasma leakage. Sensory neurons innervate vasculature/lymphatics and regulate inflammation and permeability through the release of signaling molecules that can promote tumor progression across multiple cancers. It remains unstudied how KSHV infected ECs contribute to changes in sensory neurons that innervate the vasculature/lymphatics contributing to neuronal activity changes. We hypothesize that KSHV infection alters ECs cellular communication remodeling sensory neuron communication and function contributing to disease progression. In this study, we utilized single- and bulk cell sequencing datasets to generate predicted communication networks between sensory neurons and ECs within the KS lesion. To identify the neuronal subtype most likely involved, we analyzed subtype-specific likelihood across the predicted communication network, identifying A fiber peptidergic sensory neurons as the strongest candidate. To validate our predicted communication axis, we exposed primary mouse neurons to conditioned media from KSHV-infected ECs and will assess changes in neuropeptide secretion, neurite sprouting, and neuronal Ca<sup>2+</sup> activity. Preliminary we have established a primary sensory neuron model, confirmed by  $\beta$ III-tubulin staining, and demonstrated that conditioned media from uninfected cells does not alter neuronal cell number, providing a novel platform to model KSHV-neuron interactions. These findings may uncover previously unrecognized mechanisms of KSHV-neuron communication that contribute to KS pathogenesis.

## KSHV Lytic Infection Requires and Modulates Host Nucleotide Metabolism

Fatima Hisam, Erica Sanchez

The University of Texas at Dallas, Richardson, United States

### Abstract

Kaposi's Sarcoma Herpes Virus (KSHV) is the etiological agent of Kaposi's Sarcoma (KS), known to cause metabolic stress in infected host cells. KSHV undergoes latent and lytic infection while reprogramming host metabolic pathways for efficient viral replication and infectious virion production. Previous viral-induced metabolomic studies show a dramatic increase in metabolite levels, significantly exceeding those of a normal cell. Metabolic pathways altered during latent KSHV infection were similar to those altered in cancer cells. While significant metabolomic research has been done on the latent infection, the metabolic alterations during lytic KSHV infection need further exploration. Here, we report a time-course metabolomics study conducted in the iSLK.BAC16 cells to compare latent and lytic KSHV infection. Our LCMS data show that amino acid, central carbon, and nucleotide metabolic pathways are highly dysregulated, with purine and pyrimidine metabolism among the most affected pathways. Almost all deoxynucleotides, building blocks of DNA, were elevated at 36 hrs post induction (hpi), while the triphosphate species remained elevated at 48 hpi, suggesting induced nucleotide metabolism over the course of KSHV lytic infection. Furthermore, treatment with methotrexate (MTX), a folate antagonist that inhibits nucleotide synthesis by reducing tetrahydrofolate cofactors, significantly downregulated KSHV late lytic viral gene expression, but not latent and early lytic gene expression. Viral genome replication and extracellular virion production were also significantly reduced upon reactivation after MTX treatment. Overall, our study demonstrates that inhibition of nucleotide metabolism during lytic gammaherpesvirus infection reduces productive infection and thus represents a potential therapeutic antiviral target.

## **The Role of G3BPs in Viral Replication and Translation**

Thu Nguyen, Diyun Sun, Jared Bard

Texas A&M University, College Station, United States

### **Abstract**

Chikungunya virus (CHIKV), a mosquito-borne alphavirus, relies on host factors to support viral replication. G3BP1/2, a central stress granule (SG) protein, is required for efficient CHIKV replication, but how it supports the viral life cycle remains unclear. Here, we investigate whether G3BP1/2 promotes CHIKV genomic replication and how this subsequently alters viral translation. Using a G3BP1/2 inhibitor (FAZ) that disrupts interactions with viral and SG-associated proteins, we will assess CHIKV replication and translation using qPCR, polysome profiling, imaging, and viral titers. We hypothesize that G3BP1/2 promotes CHIKV genomic replication, which in turn drives changes in viral and cellular translation during infection. These studies will define how G3BP1/2-dependent replication of CHIKV contributes to translational reprogramming during viral infection.

## Immune and bat cell lines survive contemporary Oropouche virus infection

Bryce Nielson<sup>1,2,3</sup>, Alexander Kneubehl<sup>1,2</sup>, Daniel Rehm<sup>1,2</sup>, Lucas Walther<sup>1,2</sup>, Shannon Ronca<sup>1,2,4</sup>

<sup>1</sup>Department of Pediatrics, Division of Tropical Medicine, Baylor College of Medicine and Texas Children's Hospital, Houston, United States. <sup>2</sup>William T. Shearer Center for Human Immunobiology, Texas Children's Hospital, Houston, United States. <sup>3</sup>Cancer and Cell Biology Graduate Program, Graduate School of Biomedical Sciences, Baylor College of Medicine, Houston, United States.

<sup>4</sup>Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, United States

### Abstract

Oropouche virus (OROV) is a negative-sense RNA virus endemic to the Caribbean and Central and South America. The 2024 outbreak caused by a reassortant virus resulted in the first documented human deaths caused by OROV, highlighting its emergence as a significant public health threat. This threat is coupled with a lack of information on mechanisms used by reassortant OROV to infect cells and cause severe disease. To begin characterizing OROV pathogenesis, we evaluated viral replication in epithelial, fibroblast, and immune cell lines of rodent, bat, non-human primate, and human origins. We infected cells with high and low multiplicities of infection (MOIs), observed cell growth, and collected supernatant over time. Real-time PCR was used to identify viral replication. Lines such as A549 (human lung adenocarcinoma) and CaPSM-K-SV40 (Seba's short-tailed bat kidney), had low to no viral replication while Vero CCL-81 (African green monkey kidney), CHO-K1 (Chinese hamster ovary) and NIH-3T3 (mouse fibroblast) cell lines had high levels of viral replication especially at low MOI treatments. Neither high nor low MOI treatments induced death in CaPSM-K-SV40 cells, while low MOI treatments were tolerated in THP-1 monocytes. Permissiveness and susceptibility of CCL-81 cells was consistent with previous reports. These data provide preliminary evidence of cell type- and species-specific susceptibility to OROV infection and serve as a foundation for hypothesis generation to shape subsequent investigations. Future studies will expand the types of cells represented as well as investigate cell specific traits leading to permissiveness and susceptibility.

## **vGPCR-Induced ANO1 Regulates Apoptosis and Infectious Virion Production During KSHV Reactivation**

Anisha Reddy Konakalla, Erica L Sanchez

The University of Texas at Dallas, RICHARDSON, United States

### **Abstract**

Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic gammaherpesvirus that causes Kaposi's sarcoma and other malignancies. During lytic reactivation, KSHV manipulates host signaling pathways to promote viral replication and cell survival. Here, we investigated the role of the calcium-activated chloride channel ANO1 (TMEM16A) in KSHV infection and its regulation by the viral G protein-coupled receptor (vGPCR). Transcriptomic analysis of vGPCR-expressing endothelial cells identified ANO1 as a significantly upregulated host factor, which was validated by RT-qPCR. ANO1 expression was also increased during KSHV lytic reactivation, while depletion of vGPCR reduced ANO1 expression, supporting vGPCR-dependent regulation of ANO1. Functionally, ANO1 knockdown increased apoptosis in vGPCR-expressing endothelial cells under serum-starved conditions, and inhibition of caspase activity rescued cell viability. During KSHV lytic reactivation, genetic depletion or pharmacological inhibition of ANO1 increased late apoptosis and enhanced expression of multiple KSHV lytic genes. Furthermore, loss of ANO1 function increased the production of infectious virions. Together, these findings identify ANO1 as a host factor linking vGPCR signaling to cell survival and KSHV lytic infection. Our results suggest that vGPCR-mediated induction of ANO1 promotes host cell survival while modulating progression of the KSHV lytic cycle, revealing a previously unrecognized role for ANO1 in KSHV-host interactions and highlighting ANO1 signaling as a potential therapeutic target in KSHV-associated disease.

## HIV-1 drives specific metabolism in primary human CD4+ T cells

Emma Winn<sup>1</sup>, Anjali Venna<sup>1</sup>, Ujjwal Rathore<sup>2</sup>, Robyn Kaake<sup>2</sup>, Erica Sanchez<sup>1</sup>

<sup>1</sup>University of Texas at Dallas, Richardson, United States. <sup>2</sup>Gladstone Institutes, San Francisco, United States

### Abstract

Human Immunodeficiency virus 1 (HIV-1) is an incurable retrovirus that infects around 1 million people every year. During infection, HIV-1 alters the host cell machinery to support viral replication, including cell cycle, cell survival, and host cell metabolism. There are still knowledge gaps in the specific alterations, and by understanding how HIV-1 alters host cell metabolism to provide the necessary building blocks and energetic molecules to fuel viral replication, we have the potential to identify novel targets for treatment development. The central hypothesis of our study was that there are metabolic alterations in HIV-1 infected CD4+ T cells that have not been explored. For this study, primary human CD4+ T cells were either HIV-1 or mock-infected. The intracellular metabolites were extracted and analyzed by targeted metabolomics, using LC-MS/MS. Briefly, we detected 161 unique metabolites involved in central carbon, amino acid, and nucleotide metabolism. Our data show some previously described metabolic alterations, such as elevated glycolytic intermediates and glutamine levels in HIV-1 infected samples, as well as some alterations that are unique compared to previous literature, such as in TCA cycle and amino acid metabolites. Creating a network of our altered metabolites with protein-protein interactions between HIV-1 proteins and host proteins, and HIV dependency or restriction factors, we highlight potentially novel interactions between HIV-1 proteins and altered host cell metabolites. In conclusion, our data demonstrate how HIV-1 alters host cell metabolism in CD4+ T cells, and provides next steps of study for determining specific mechanisms of alteration.

## **Medical Microbiology Group 1 Graduate Student Posters**

## **Decoding Bovine MG53 Protein: Tissue Expression, Structural Characterization, Recombinant Protein Production and Putative Role in PPAR $\alpha$ -Mediated Intestinal Barrier Protection**

Ahmad Ali<sup>1</sup>, Oscar Javier Benitez<sup>2</sup>, Lauren Gollahon<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Texas Tech University, Lubbock, United States. <sup>2</sup>Department of Veterinary Sciences, Texas Tech University, Lubbock, United States

### **Abstract**

MG53 (Mitsugumin 53), a member of the tripartite motif-containing protein family, is a key mediator of cell membrane damage repair and tissue regeneration. Its therapeutic potential has been extensively studied in several rodent and human diseases. However, MG53 expression, and functional relevance in bovine remain largely unexplored. In bovine, gut health is central to productivity as it supports nutrient absorption, immune regulation and pathogen exclusion. Disruption of intestinal-barrier function increases permeability, allowing luminal pathogens, toxins, and inflammatory molecules to enter circulation and contribute to disease susceptibility and reduced productivity. In this study, we used an integrated tissue-to-function approach to begin investigating the hypothesis that MG53 supports intestinal epithelial integrity, thereby mitigating pathologies associated with leaky gut syndrome.

Endogenous bovine MG53 expression was evaluated by Western in skeletal muscle, heart, kidney, lung, and gastrointestinal tissues. Expression was highest in skeletal muscle. Comparative sequence analysis, phylogenetic reconstruction, and structural modeling identified conserved RING finger, B-box, and SPRY domains, relevant to protein stability, and oligomerization.

These findings guided production of bovine recombinant MG53. The MG53 coding sequence was cloned into an affinity-tagged bacterial expression plasmid and expressed in a bacterial host. Initial expression and purification result informed codon optimization to improve translational efficiency, soluble expression, and purified protein yield. Protein purity was assessed by SDS-PAGE and Western. Purified recombinant MG53 will enable future investigation of MG53 mechanism of action in intestinal epithelial repair in bovine.

## Antimicrobial resistance and efficacy of repeat intravesical VesiX therapy in the management of recurrent urinary tract infection

Nimna Amarasinghe<sup>1</sup>, Philippe E. Zimmern<sup>2</sup>, Nicole J. De Nisco<sup>1,2</sup>

<sup>1</sup>The University of Texas at Dallas, Richardson, United States. <sup>2</sup>The University of Texas Southwestern Medical Center, Dallas, United States

### Abstract

Recurrent urinary tract infections (rUTIs) are common among postmenopausal women. rUTIs are treated with repeated antibiotic courses, driving the emergence of resistant uropathogens. Intravesical treatments are a promising alternative as they provide localized therapy without systemic exposure. VesiX, a novel formulation of the broad-spectrum antiseptic Cetylpyridinium chloride, has shown activity against uropathogenic bacteria both extracellularly and intracellularly. However, its efficacy as an intravesical treatment for rUTI has yet to be evaluated. This clinical study investigates the urinary microbiological profiles of postmenopausal women receiving repeated VesiX bladder instillations and evaluates whether repeated exposure promotes VesiX resistance.

Eight women with antibiotic refractory rUTI were administered four VesiX bladder instillations each 2-3 weeks apart. Urine was collected before and after each instillation and cryopreserved in 50% glycerol. Urine was plated on CHROMagar Orientation to facilitate uropathogen cultivation, followed by species confirmation via Sanger sequencing of 16S rRNA amplicons. VesiX minimum inhibitory and bactericidal concentrations were determined by broth microdilution in Cation-adjusted Mueller Hinton Broth.

By the end of the four VesiX instillations, 75% of patients had either no cultivable uropathogens or reduced uropathogen abundance. Recovered species included *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus spp.*, *Streptococcus agalactiae*, *Proteus mirabilis*, and *Staphylococcus spp.* No uropathogens were detected in post-instillation urine. All isolates were susceptible to VesiX at concentrations at least 4X lower than the therapeutic concentration. No uropathogens gained resistance to VesiX after repeated exposure. These findings suggest VesiX may be a promising intravesical rUTI therapy, but this must be validated in a larger study population.

## Bicarbonate promotes *Pseudomonas aeruginosa* persistence during macrophage infection despite enhanced bacterial uptake

Ravali Arugonda

The University of Texas at Dallas, Richardson, United States

### Abstract

*Pseudomonas aeruginosa* is one of the ESKAPE pathogens known to cause chronic infections. After the initial colonization, *P. aeruginosa* persists within the host, develops antibiotic resistance, and eventually kills the patient. Previous studies have shown that bicarbonate ( $\text{HCO}_3^-$ ), an important component that maintains host homeostasis, has been shown to improve pathogen clearance. However, not much is known about the complex interactions between the bacteria,  $\text{HCO}_3^-$ , and host immune cells. Our preliminary data on a THP-1 macrophage infection assay with *P. aeruginosa* clinical isolate P4 suggest that  $\text{HCO}_3^-$  increased P4 uptake by macrophages but did not translate to improved clearance. Instead, P4 exhibited greater intracellular persistence in the presence of  $\text{HCO}_3^-$ . We have also observed that  $\text{HCO}_3^-$  increased infection-associated ROS production. Transcriptional analysis of P4 with  $\text{HCO}_3^-$  exposure revealed extensive physiological remodeling that includes oxidative and nitrosative stress responses.

Our findings reveal that  $\text{HCO}_3^-$  uncouples P4 uptake and ROS production from effective killing by differentiated THP-1 cells. We hypothesize that  $\text{HCO}_3^-$  acts as a signal that restricts growth and that the physiology is reprogrammed in a way to adapt to a macrophage-derived antimicrobial stress. This  $\text{HCO}_3^-$  dependent phenotype may be contributing to persistence of P4 in differentiated THP-1 cells despite the increased phagocytosis and ROS levels. We aim to elucidate bacterial pathways that support intracellular persistence. Identifying this could provide new targets for improving chronic *P. aeruginosa* infections.

## Clostridiales Disruption Shapes Longitudinal Short-Chain Fatty Acid Dynamics During Antibiotic Therapy

Hossana Ayele<sup>1</sup>, Abdulwhab Shremo Msdi<sup>2</sup>, Khurshida Begum<sup>2</sup>, Chenlin Hu<sup>2</sup>, M. Jahangir Alam<sup>2</sup>, Taryn Eubank<sup>2</sup>, Sigmund J. Haidacher<sup>3,4</sup>, Thomas D. Horvath<sup>3,4</sup>, Kevin W. Garey<sup>2</sup>, Blake M. Hanson<sup>1</sup>

<sup>1</sup>UTHealth, Houston, United States. <sup>2</sup>University of Houston, Houston, United States. <sup>3</sup>Baylor College of Medicine, Houston, United States. <sup>4</sup>Texas Children's Hospital, Houston, United States

### Abstract

Short-chain fatty acids (SCFAs) are microbial fermentation products that support gut homeostasis, epithelial integrity, and colonization resistance. Linear SCFAs dominate the fecal SCFA pool and mediate beneficial immune and metabolic regulation. Notably, members of the Clostridiales order produce the majority of gut-derived linear SCFAs and are vulnerable to antibiotic disruption. This study sought to identify microbial taxa associated with longitudinal changes in SCFAs during antibiotic therapy. Healthy volunteers aged 18-40 participated in a phase 1, nonblinded, randomized clinical trial and received a 10-day course of oral moxifloxacin, vancomycin, or omadacycline. Stool samples were collected at baseline (D0), during therapy (D1-D10), and end of therapy (D13 and D30). Microbial characterization of fecal samples included 16S V1-V3 rRNA sequencing. SCFAs were quantified using LC-MS/MS and normalized to stool sample weight. SCFAs evaluated included linear (acetic, propionic, butyric, valeric acid) and branched (isobutyric, isovaleric, and 2-methylbutyric acid). Maaslin3 linear mixed-effects modeling was used to assess associations between microbial orders and SCFA concentrations while accounting for repeated sampling. Baseline SCFA concentrations were comparable across antibiotic arms; however, SCFA depletion differed during therapy. Vancomycin produced the greatest disruption, with average trough reductions of ~90-fold for linear SCFA and 55-fold for branched relative to baseline. Clostridiales abundance positively associated with linear SCFA concentrations and declined sharply in the vancomycin arm, with moderate and minimal reductions observed with omadacycline and moxifloxacin, respectively. Antibiotic-associated reductions in Clostridiales corresponded to decreased linear SCFAs, underscoring their importance in maintaining microbial metabolic function during antimicrobial exposure.

## Molecular determinants of *Candida auris* skin colonization as targets for decolonization

Katy Ayers, Michael Lorenz

University of Texas MD Anderson Cancer Center UT-Health Houston Graduate School of Biomedical Sciences, Houston, United States

### Abstract

*Candida auris* is an emergent multidrug-resistant fungal pathogen capable of asymptomatically colonizing human skin, creating a persistent reservoir for transmission in healthcare settings. Despite the importance of skin colonization to *C. auris* epidemiology, the molecular determinants that enable adaptation and persistence within this niche remain incompletely defined. The skin presents a unique combination of environmental challenges including elevated osmolarity and limited nutrient availability, requiring *C. auris* to adapt its interactions with the host and its utilization of available resources. We hypothesize that persistent skin colonization requires coordinated adaptation to these physical, chemical, and nutritional pressures through pathogen-specific mechanisms governing adhesion, osmotic stress tolerance, and alternative carbon metabolism, and that disruption of these adaptations will compromise skin fitness and reveal vulnerabilities for decolonization. To identify genetic determinants of skin-associated fitness, we are screening a *C. auris* transcription factor mutant library in artificial skin medium to identify regulatory systems governing adaptation. Candidate regulators identified through this approach will be characterized for their roles in halotolerance and adaptation to the skin environment. Complementary studies will define the contributions of specific adhesins to retention of skin-associated substrates and evaluate fatty acid metabolism as a targetable metabolic dependency under skin-like conditions. Together, these studies will establish how *C. auris* adapts to the skin niche at the genetic and mechanistic levels and determine whether these adaptations can be exploited therapeutically. Defining pathogen-specific requirements for skin persistence may provide new strategies to reduce colonization, interrupt nosocomial transmission, and prevent subsequent invasive infection.

## Exploration of the *lacGDRA* operon in cancer-derived *Lactobacillus iners*

Laurel Boatright<sup>1</sup>, Michael Holder<sup>1</sup>, Martha Zepeda-Rivera<sup>1</sup>, Molly Blue El Alam<sup>1</sup>, Chia-Chi Chang<sup>1</sup>, Adilene Olvera<sup>1</sup>, Nadim Ajami<sup>1</sup>, Pablo Okhuysen<sup>1</sup>, Christopher Johnston<sup>1</sup>, Lauren Colbert<sup>2</sup>

<sup>1</sup>UT MD Anderson Cancer Center, Houston, United States. <sup>2</sup>Duke University, Durham, United States

### Abstract

**Background:** *Lactobacillus iners* (*L. iners*) is a highly prevalent vaginal microbe and has been associated with poor outcomes in patients with cervical cancer. Our previous work identified *lacG* and the *lacGDRA* operon as a potential commonality within cancer-derived (CC) *L. iners*. The purpose of this study was to determine the frequency of *lacGDRA* in CC versus non-cancer derived (NC) *L. iners* isolates, and to evaluate its relationship with lactose-dependent growth under conditions relevant to the tumor microenvironment (TME). **Methods:** This study included MDACC CC isolates (n=12), MDACC NC isolates (n=3), and public *L. iners* genomes (n=21). RNA sequencing was performed in triplicate in NYCIII media. Growth in lactose-substituted media was assessed in anoxia (0% O<sub>2</sub>) and tumor-relevant hypoxia (1% and 5% O<sub>2</sub>) and monitored via OD<sub>600</sub>. **Results:** 60% (8/14) of CC and 50% (11/22) of NC genomes possessed *lacG* and a complete *lacGDRA* operon (p = 0.6756). 92% (33/36) of all genomes possessed *lacD*, *lacR*, and *lacA*. All MDACC isolates expressed *lacD*, *lacR*, and *lacA* and *lacG* was expressed in isolates with a *lacG* gene. *lacG*-positive isolates grown in lactose-substituted media in anoxia reached significantly higher OD<sub>600</sub> than *lacG*-negative isolates (p < 0.0001). Hypoxia did not significantly impact growth of *lacG*-negative isolates in lactose-substituted media (p=0.2460), but significantly reduced growth of *lacG*-positive isolates (p < 0.0001). **Conclusions:** Together, these data suggest that *lacG* influences nutrient utilization in depleted environments, and that this effect that may vary across *L. iners* isolates and be shaped by oxygen in the cervical TME.

## Characterization of a Novel Murine Model of Severe Trauma-Associated Mucormycosis

William Carpenter<sup>1</sup>, Paige Diaz<sup>2</sup>, Lizette Rios<sup>1</sup>, Juquan Song<sup>1</sup>, Joseph Wenke<sup>1</sup>, Alison Coady<sup>1</sup>

<sup>1</sup>University of Texas Medical Branch, Galveston, United States. <sup>2</sup>MD Anderson Cancer Center, Houston, United States

### Abstract

Severe traumatic injury results in systemic immune dysregulation that predisposes a patient to serious invasive fungal infections which increase risk of delayed wound healing, amputation, and mortality. Infections caused by Mucorales molds are particularly lethal, characterized by hyphal angioinvasion, rapid dissemination, and broad resistance to first-line antifungals. However, the host-pathogen interactions that drive disease progression in the setting of trauma remain poorly defined, limiting the development of new therapeutic strategies. This problem is confounded by the lack of accessible preclinical models of severe trauma-related fungal infection.

To address this, we've developed a murine model that combines a scald burn, extremity injury, and fungal infection to study the effects of severe trauma on Mucorales pathogenesis. Immunocompetent C57BL/6J mice receive a scald burn covering 15-20% of their total body surface area, followed by a penetrating skeletal muscle injury via cryoinjury. The resulting extremity wound is contaminated with a standardized inoculum of *Rhizopus oryzae* spores resuspended in a biodegradable thermo-sensitive polymer matrix. Mice subjected to burn and muscle injury succumb to infection with germinated *R. oryzae* spores in 7 days, in contrast to infected mice given a cryoinjury alone.

Current work is focused on further optimizing molecular quantification of fungal burden by digital droplet PCR and flow cytometric profiling of systemic immune responses. Ultimately, this model will allow us to identification of precise cellular and molecular mechanisms of pathogenesis and support the testing of novel therapeutics.

## How to Tune your Membrane: Turnover of Lysine-modified lipids in *Streptococcus agalactiae*

Priya Christensen<sup>1</sup>, Dur Al Hamami<sup>1</sup>, Luke Joyce<sup>2</sup>, Bryan Estrada<sup>1</sup>, Kelly Doran<sup>2</sup>, Brandon Kim<sup>1</sup>, Kelli Palmer<sup>1</sup>

<sup>1</sup>The University of Texas at Dallas, Richardson, United States. <sup>2</sup>University of Colorado Anschutz School of Medicine, Aurora, United States

### Abstract

Bacteria must adapt quickly in response to external stressors, often by modifying their membrane charge. Group B *Streptococcus* (GBS) modifies its membrane through the multiple peptide resistance factor (MprF), which generates aminoacylated lipids. GBS MprF synthesizes lysyl-glucosyl-diacylglycerol (Lys-Glc-DAG) and lysyl-phosphatidylglycerol (Lys-PG). These cationic lipids protect against external stressors, but little is known about how the membrane returns to normal after the stressors are gone. We hypothesize that the alpha-beta hydrolase, AhyD, removes lysine from Lys-lipids, thus bringing the membrane back to homeostasis. Indeed, we observed an increase in Lys-lipids in an *ahyD* deletion strain compared to the wild-type COH1 strain. Moreover, the *ahyD* deletion strain was less susceptible to daptomycin, an antibiotic for which increased Lys-PG confers reduced susceptibility in other Gram-positive pathogens. Prior work found that *mprF* deletion significantly compromised invasion of human cerebral microvascular endothelial cell (hCMEC) by GBS. We hypothesized that the *ahyD* mutant would have increased invasion of hCMEC and induced brain-like endothelial cells. Unexpectedly, we observed no difference in invasion. Instead, we observed an increase in pigmented colonies recovered post-invasion with the *ahyD* mutant, indicating increased expression of the GBS cytolyisin (encoded by the *cyl* operon). Under normal conditions, the *ahyD* mutant does not have altered *cyl* operon gene expression; rather, it appears that the spontaneous emergence of *cyl* hyper-expressing mutants is higher post-invasion with the *ahyD* mutant. These results indicate that *ahyD* plays an important role in maintaining GBS cell membrane homeostasis and that its loss may increase the heterogeneity of GBS during infection.

## Defining Contribution of Progesterone Signaling to Group B Streptococcal Interaction at the Blood-Brain Barrier

Darla Delozier<sup>1</sup>, Katelin Stilen<sup>1</sup>, Nadine Vollmuth<sup>2,3</sup>, Jacob Wood<sup>4</sup>, Robert McCullumsmith<sup>4</sup>, Kathryn Seely<sup>1</sup>, Brandon Kim<sup>1</sup>

<sup>1</sup>University of Texas at Dallas, Richardson, United States. <sup>2</sup>University of Alabama, Tuscaloosa, United States. <sup>3</sup>University of Würzburg, Würzburg, Germany. <sup>4</sup>University of Toledo, Toledo, United States

### Abstract

*Streptococcus agalactiae* (Group B Streptococcus, GBS) is a Gram-positive opportunistic pathobiont and is the leading cause of neonatal meningitis worldwide. We have previously shown that estrogen signaling can contribute to GBS interaction with the blood-brain barrier (BBB) contributing to meningitis. Examination of large deidentified datasets have revealed an additional role for the sex hormone progesterone in invasive GBS disease with an increased risk of neonatal GBS infection from women undergoing perinatal progesterone hormone treatment. Therefore, we hypothesize that progesterone may contribute to bacterial interaction with the BBB. To model the BBB, induced pluripotent stem cell-derived brain-like endothelial cells (iBECs) and immortalized cerebral microvascular endothelial cells (hCMEC/D3s) were treated with progesterone prior to GBS infection in order to further understand the relationship between GBS infection and progesterone signaling at the BBB. Both cell lines show a significant increase in GBS adherence with an increase in progesterone concentration, suggesting that progesterone signaling could contribute to the susceptibility of the BBB to GBS infection. Barrier integrity is decreased in progesterone-treated cells as well, as demonstrated by a decrease in both Trans Endothelial Electrical Resistance (TEER) and efflux activity. The results of further protein and mRNA quantification of tight junctions, efflux transporters, and phosphorylated signaling proteins will allow us to identify the specific pathways contributing to the weakening of the BBB and develop strategies for prevention of neonatal GBS infection as a whole.

## Genetic Dissection of Virulence and Host Interactions in *Candidozyma auris*

Kimberly Garcia<sup>1,2</sup>, Melissa Martinez<sup>1,2</sup>, Michael Lorenz<sup>1,2</sup>

<sup>1</sup>University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, Houston, United States. <sup>2</sup>University of Texas Health Science Center, McGovern Medical School, Department of Microbiology and Molecular Genetics, Houston, United States

### Abstract

*Candidozyma auris* is an emerging fungal pathogen notable for multidrug resistance, persistence on skin and surfaces, and tolerance to disinfectants. These traits complicate infection control and are associated with mortality rates approaching 60% in immunocompromised patients with invasive candidiasis. Despite its clinical significance, the genetic basis for its virulence and resilience remains unclear. Much of what is known about fungal virulence and host interactions comes from studies on *Candida albicans*, a related but genetically and physiologically distinct species. While traditional animal models have provided valuable insight into fungal pathogenesis, they are not optimized for large-scale genetic screening. A rapid, high-throughput screening approach is needed to expand our understanding of *C. auris* virulence mechanisms. To address this, we developed a *Caenorhabditis elegans* infection model for *C. auris* and adapted it for high-throughput use. This complementary approach offers scalability, cost-effectiveness, and conservation of innate immune pathways relevant to fungal infection. Because *C. auris* is haploid, it is particularly well suited for forward genetics. Our lab generated a T-DNA insertion mutant library using *Agrobacterium tumefaciens*-mediated transformation in a *C. auris* clinical isolate. Initial screening identified multiple mutants with reduced virulence, including hits in genes involved in DNA replication, adhesion, chromatin regulation, cell wall and membrane maintenance, and protein kinase activity. Disruption of several uncharacterized open reading frames also resulted in attenuated virulence, suggesting they may encode novel virulence factors. Ongoing work includes secondary screening and generation of clean deletion mutants to confirm gene function and further define their roles in *C. auris* pathogenesis.

## ***Caenorhabditis elegans* Slow Killing Recapitulates Chronic *Pseudomonas aeruginosa* Infection and Reveals Mediators of Host-Pathogen Interactions**

Sadie Gaskins, Liyang Zhang, Donghoon Kang, Qi Xu, Natalia Kirienko  
Rice University, Houston, United States

### **Abstract**

*Pseudomonas aeruginosa* is a leading cause of healthcare-associated infections, especially in immunocompromised patients and individuals with cystic fibrosis (CF). *P. aeruginosa* commonly causes chronic infections within the respiratory tracts of patients with CF. These infections are marked by a shift from the production of acute virulence factors (secreted toxins) to colonization (biofilm formation) and persistence within the host.

To better understand the transcriptomic changes that occur during this shift within the context of host-pathogen interactions, we took advantage of a whole-organism model system. Using an established *Caenorhabditis elegans* – *P. aeruginosa* colonization model, we identified bacterial genes differentially regulated during establishment of the infection of the *C. elegans* intestine. By comparing this transcriptomic signature to those from *P. aeruginosa* from CF sputum samples and an *ex vivo* porcine lung biofilm model, we demonstrated that Slow Killing partially recapitulates chronic lung infection. Notably, we observed the upregulation of lipid A and exopolysaccharide (Pel, alginate) biosynthetic genes within the nematode host and downregulation of acute virulence pathways including quorum-sensing, phenazine production, toxin secretion, and siderophore-mediated iron uptake. Further characterization of these differentially-regulated genes revealed key bacterial pathways, including quorum-sensing and the PhoPQ regulatory network, that impacted host survival during infection. These results demonstrate that the *C. elegans* Slow Killing model is a highly accessible tool for studying host-pathogen interactions and benchmark it against clinical samples.

## ***Staphylococcus aureus* thymidine-dependent small colony variants induce enhanced inflammation through remodeled cell wall components**

Pratyusha Gorige, Qing Tang

University of Texas at Arlington, Arlington, United States

### **Abstract**

Individuals with cystic fibrosis (CF), who have thickened mucus and dysregulated lung immune microenvironments due to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, are highly susceptible to *Staphylococcus aureus* lung infections. *S. aureus* thymidine-dependent small-colony variants (TD-SCVs), which carry inactivate mutations in thymidylate synthase (ThyA), are frequently identified in the lungs of CF children with prolonged anti-folate antibiotic treatment. *S. aureus* TD-SCV infection is associated with more severe lung disease and worse clinical outcomes in CF children, with underlying mechanisms unclear. To investigate how TD-SCV infection modulates host immune response, we generated a laboratory *S. aureus* TD-SCV strain,  $\Delta thyA$ . We found that both  $\Delta thyA$  and LTA extracted from  $\Delta thyA$  grown under low-thymidine conditions induced higher pro-inflammatory cytokine expression in primary bone marrow-derived macrophages (pBMDMs) than  $\Delta thyA$  grown under high-thymidine conditions or the wild-type strain. RNA-seq analysis of pBMDMs stimulated with LTA extracted from  $\Delta thyA$  and WT revealed that LTA from  $\Delta thyA$  specifically induced elevated expression of NF- $\kappa$ B-dependent pro-inflammatory cytokines. We further found that this elevated gene expression was primarily TLR2–MyD88-dependent. Moreover, clinical TD-SCV isolates recapitulated the phenotypes observed in the  $\Delta thyA$  strain. Together, these findings identify LTA-mediated TLR2–MyD88–NF- $\kappa$ B signalling as a mechanism by which *S. aureus* TD-SCVs enhance host inflammation upon thymidine limitation. Ongoing LTA structural characterization and mouse infection studies will define thymidine availability-dependent LTA alterations and evaluate pharmacological blockade of TD-SCV-induced inflammation to reduce CF-associated lung pathology.

## **Medical Microbiology Group 2 Graduate Student Posters**

## Characterization of SAP as a Novel Virulence Factor in Group B *Streptococcus* Bacterial Meningitis

Brice Gorman<sup>1</sup>, Cody Cutts<sup>1</sup>, Melanie Higgins<sup>2</sup>, Brandon Kim<sup>1</sup>

<sup>1</sup>The University of Texas at Dallas, Richardson, United States. <sup>2</sup>The University of Alabama, Tuscaloosa, United States

### Abstract

Group B *Streptococcus* (GBS) is the leading cause of neonatal meningitis worldwide. Bacterial meningitis often occurs from the disruption of the blood brain barrier, formed mostly of specialized brain endothelial cells (BECs). To identify genes in GBS possibly implicated in virulence, dual-RNAseq analyzing expression during infection of BECs was performed. A cell wall anchored glycoside hydrolase, *Streptococcus agalactiae* pullulanase (SAP), was shown to be up-regulated. Genetic knock-out of SAP ( $\Delta$ SAP) showed a significant reduction of adherence and invasion in both the induced pluripotent stem cell derived BEC (iBEC) model and the human cerebrovascular endothelial cell (hCMEC/D3) model when compared to wild-type. Additionally, mice infected with  $\Delta$ SAP tended towards longer survival and had significantly reduced bacteria in the brain compared to mice infected with wild-type GBS. Structural comparison of SAP to the experimentally characterized *Streptococcus pneumoniae* Pullulanase A (spuA) showed high degree of similarity, though varying in residues found within a carbohydrate binding motif (CBM) and the catalytic module (CM). Additionally, an intrinsically disordered domain near the N-terminus of SAP shows little to no sequence homology to known proteins outside of GBS. Future endeavors will include recombinant protein production, in-frame domain knock-outs, and point mutations in both recombinant and endogenous SAP to deaden the CBMs and CM.

## **Type VII Secretion System Effector Regulates Pil3 Expression and Adherence in *Streptococcus gallolyticus subsp. gallolyticus*.**

Rian Hernandez, Jyoti Tamang, Sarah Woodfin, Yi Xu

Texas A&M University Institute of Biosciences and Technology, Houston, United States

### **Abstract**

*Streptococcus gallolyticus subsp. gallolyticus* (Sgg) is an opportunistic pathogen associated with infective endocarditis and colorectal cancer (CRC). Sgg interaction with the colonic epithelium is a critical early step in Sgg pathogenesis. The Type VII Secretion System (T7SS) mediates Sgg interaction with the colonic epithelium at multiple levels. However, the mechanisms underlying T7SS's roles remain unclear.

Here, we examined a panel of deletion mutants of T7SS to further pinpoint the contributions by specific T7SS effectors. We found that loss of Sgg511, a T7SS-secreted LXG-domain containing protein, resulted in significantly reduced adherence to CRC cells, loss of ability to stimulate CRC cell proliferation, and impaired gut colonization.

Transcriptomic analysis revealed significant downregulation of genes within the pil3 operon, which encodes pilus Pil3, following disruption of Sgg511 or T7SS function. Consistent with these findings, flow cytometry using anti-Pil3 antibodies demonstrated altered Pil3 surface protein expression in  $\Delta$ Sgg511, exhibiting a biphasic pattern containing Pil3-high and Pil3-low expressing subpopulations that is distinct from the isogenic wild type parent strain. In contrast, this altered expression pattern was not observed for Pil1, suggesting a model in which Sgg511 promotes Sgg adherence, at least in part by maintaining Pil3 expression and surface presentation.

Both T7SS and pili are important for bacterial virulence. The findings here provide the first experimental evidence for a regulatory connection between a T7SS-secreted effector and pili expression. Ongoing studies are investigating how Sgg511 regulates Pil3 expression and the role of Pil3 in Sgg511-mediated phenotypes.

## Role of Beta-2 Adrenergic Receptor Signaling in *Streptococcus pneumoniae* Infection of the Blood Brain Barrier

Taryn Keyzer, Brandon Kim

University of Texas at Dallas, Richardson, United States

### 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). *Streptococcus pneumoniae* (pneumococcus) is the leading cause of bacterial meningitis world-wide; however, the mechanisms through which the BBB fails during pneumococcal meningitis remain poorly understood. 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). A human population-based study determined that patients with the single nucleotide polymorphism (SNP) rs1042714 in the beta-2 adrenergic receptor (B2AR) were more susceptible to pneumococcal meningitis than patients with wild type B2AR. We therefore hypothesize that B2ARs play an important role in pneumococcal interaction and disruption of the BBB. To initially test this hypothesis, we employed well-described, FDA-approved beta-blockers. Treating iBECs with the pan beta-blocker propranolol resulted in a significant decrease in pneumococcal invasion; however, treatment with a B1AR inhibitor had no effect. These preliminary data support our hypothesis that B2AR contributes to bacterial interaction and highly motivate further investigation of the rs1042714 SNP. Next, we knocked-in the SNP into our iPSC line and differentiated these gene-edited cells into iBECs. The SNP iBECs display increased pneumococcal adherence and invasion compared to control iBECs. Here, for the first time, we are leveraging population-based studies, CRISPR technologies, and our stem cell-based models to discover a mechanistic role for host genetic variation and its contribution to bacterial meningitis.

## Novel Small Molecule Inhibitors Directed Against Phosphoinositide Signaling Block *Nakaseomyces glabratus* Growth and Protease Secretion

Ashley Kidwell<sup>1</sup>, Carolyn Cannon<sup>1</sup>, Tatyana Igumenova<sup>2</sup>, Vytas Bankaitis<sup>1</sup>

<sup>1</sup>Texas A&M University Naresh K. Vashist College of Medicine, Bryan, United States. <sup>2</sup>Texas A&M University, College Station, United States

### Abstract

Invasive fungal infections with multiple drug resistance present a rising public health crisis that urges researchers to study novel antimycotic strategies. Secretory cargo was previously identified as an essential component of fungal pathogenesis in *Candida* model systems. Hence, pharmacologic targeting of fungal phosphoinositide (PIP) signaling, the molecular stamps of sorting membranes, are of immense interest to researchers. Previously published small molecule inhibitor backbones were well-established as ligands of *S. cerevisiae* Sec14, a phosphatidylinositol transfer protein, and crystallized as competitive inhibitors at high-resolution. Among pathogenic fungi, *N. glabratus*, formerly *Candida glabrata*, is the closest in phylogenetic relationship to *S. cerevisiae* and served as an appropriate model to begin to study the impact of this inhibitor class on pathogenesis. In the present study, novel small molecule inhibitors of fungal Sec14 were evaluated for *in vitro* antimycotic, anti-biofilm, and anti-virulence factor activity against *N. glabratus* CBS138. Inhibitors displayed potent *in vitro* minimal inhibitory concentrations (2 µg/mL) and a fungistatic effect. Small molecule inhibitors were found to halt biofilm maturation processes quantified by biomass, metabolic activity, and visualization under brightfield microscopy. Under challenge, *N. glabratus* CBS138 showed attenuated protease activity over a 10-day period. Small molecule inhibitors of fungal Sec14 thus frame a strategy to block *N. glabratus* growth and virulence secondary to protease activity *in vitro*. This study supports molecular membrane sorting through PIPs as a candidate antimycotic target pathway.

## ***Streptococcus agalactiae* inhibits Breast Cancer Resistance Protein function in brain endothelial cells**

Benjamin Klemp<sup>1</sup>, Natalie Alexander<sup>1</sup>, Alex Meyer<sup>2</sup>, Elisa Tucker<sup>2</sup>, Brandon Kim<sup>1</sup>

<sup>1</sup>University of Texas at Dallas, Richardson, United States. <sup>2</sup>University of Alabama, Tuscaloosa, United States

### **Abstract**

Infection of the central nervous system (CNS) in the form of bacterial meningitis poses a serious health threat, arising from failure of the blood-brain barrier (BBB). A causative agent of meningitis is the bacterium *Streptococcus agalactiae*, or Group B *Streptococcus* (GBS), an opportunistic pathogen that typically colonizes the genital and gastrointestinal tracts. Bacteremia and subsequent meningitis require GBS to interact with and disrupt the BBB. The BBB is comprised of brain endothelial cells (BECs) that possess efflux transporters to return exogenous materials back into circulation. One such efflux transporter is Breast Cancer Resistance Protein (BCRP), contributing to the transport barrier of the BBB through the expulsion of over 200 substrates. While other BBB properties are known to be disrupted during GBS infection of the BBB, BCRP function has not been well characterized in this context. Here, reduction of BCRP function and abundance was observed in induced pluripotent stem cell-derived BECs following GBS infection and live bacteria were necessary to observe this phenomenon. Additionally, a BCRP overexpressing cell-line is being utilized to further demonstrate the influence of GBS infection on BCRP function at the BBB.

## **Mechanisms underlying adverse neurodevelopmental outcomes in mouse offspring exposed to maternal *T. cruzi* infection in utero**

Samuel Li, Cristina Poveda, Noah Haugen, Kathryn Jones, Shelly Buffington  
Baylor College of Medicine, Houston, United States

### **Abstract**

The protozoa *Trypanosoma* (*T.*) *cruzi* causes Chagas disease, a prevalent neglected tropical disease endemic to the Americas. Chagas disease affects millions of people annually and was recently declared endemic to the United States in 2025. Chagas disease is known to cause debilitating neurological illness including meningoencephalitis, weakness, and speech disturbances, but little is known about neurological effects in offspring because of maternal infection. Recent studies in our lab have shown that in utero exposure to acute maternal *T. cruzi* infection increases risk for brain and behavioral phenotypes characteristic of genetic mouse models for neuropsychiatric disorders, specifically schizophrenia, irrespective of congenital transmission. This data suggests that the *T. cruzi*-induced MIA during pregnancy is sufficient to alter fetal brain development in ways that manifest as maladaptive behavior in adolescence and adulthood. Thus, the goal of this project is to (1) identify changes in immune signaling at the maternal-fetal interface driven by *T. cruzi* infection in pregnancy, (2) determine if the maternal immune response to *T. cruzi* is causal in the adverse neurodevelopmental outcomes in offspring, and (3) whether the maternal and/or offspring immune and behavioral phenotypes, respectively, can be reversed or at least attenuated through metagenomics-informed targeted modulation of the maternal gut microbiome in pregnancy. Overall, this project provides proof-of-concept of a model of *Tc* infection in MIA that is scalable and easily replicable to provide further insight into unexplored mechanisms of Chagas disease and prioritization of vaccination against Chagas for women of childbearing age.

## DEFINING BACTERIAL AND MATERNAL IMMUNE DETERMINANTS OF *KLEBSIELLA PNEUMONIAE* VAGINAL COLONIZATION AND VERTICAL TRANSMISSION

Mariana Lozano-Cavazos, Addison Larson, Holly Banthoover, Cilicia Nascimento, Katy Patras  
Baylor College of Medicine, Houston, United States

### Abstract

Neonatal sepsis is a major cause of neonatal morbidity and mortality worldwide, with *Klebsiella pneumoniae* among the leading bacterial causes of neonatal infection. Although maternal vaginal *K. pneumoniae* colonization has been associated with adverse pregnancy outcomes and neonatal infection, the mechanisms linking maternal reproductive tract colonization to fetal transmission and neonatal disease remain poorly understood. We developed a novel murine model of maternal vaginal *K. pneumoniae* colonization to investigate bacterial and host determinants of reproductive tract colonization, dissemination, and vertical transmission. Using VSI78, a vaginal isolate from a healthy donor, KPPR1, a well-characterized clinical isolate, and KPPR1 deletion mutants targeting *wcaJ* (capsule biosynthesis), *fimH* (type 1 fimbriae), and *clpV* (type VI secretion system), we investigated strain- and factor- specific effects on interactions with human vaginal epithelial VK2 cells and reproductive tract fitness.

VSI78 demonstrated greater cytotoxicity and adhesion to VK2 cells than KPPR1, whereas KPPR1 exhibited greater vaginal colonization and persistence *in vivo*, demonstrating distinct strain-specific phenotypes. In pregnant mice, intravaginal infection with KPPR1 resulted in maternal bacteremia and fetal reabsorption in 57% of dams. In contrast, VSI78-infected dams delivered at term but exhibited substantial vertical transmission, with *K. pneumoniae* detected in the liver and intestine of 77% of pups. Neonatal infection was associated with 70% mortality by day 1 postpartum and 100% infection and mortality by day 4 postpartum.

Together, these findings establish maternal vaginal *K. pneumoniae* colonization as a potential route of fetal and neonatal transmission and provide a tractable model to define determinants of neonatal disease.

## **Bacterial Production of Histamine as a Novel Immune Evasion Strategy of *Aeromonas dhakensis***

Blake Neil, Paul Kilgore, Atul Verma, Jian Sha, Ashok Chopra

University of Texas Medical Branch, Galveston, United States

### **Abstract**

Transcriptomic profiles of two strains of *Aeromonas dhakensis* isolated from fatal monomicrobial cases of bacteremia grown either *in vitro* or *in vivo* revealed significant upregulation of histidine decarboxylase (*hdc*) *in vivo*. This gene encodes the HDC enzyme responsible for converting histidine to histamine. While bacterial histamine is under current investigation for its role in microbial-host crosstalk, little is known of its role in the context of an acute infection. First, no genomic or transcriptional evidence was found of dedicated histamine sensing machinery in our isolates of *A. dhakensis* and this was corroborated by an inability to grow using histamine as the sole source of nitrogen and a lack of chemotactic behavior toward histamine. Next, evidence of the *hdc* gene's involvement in the production of a novel histamine-containing siderophore was observed, though growth curve assays showed this siderophore to be nonessential for survival in iron limiting conditions. Finally, we observed an attenuation of virulence when the *hdc* gene was deleted compared to the parental wild type (WT) bacteria both *in vitro* and *in vivo* and the addition of exogenous histamine to RAW 264.7 cell infection with  $\Delta hdc$  restored histamine levels and cell death rates to that observed during WT infection. A dysregulated cytokine response following infection both *in vitro* and *in vivo* with  $\Delta hdc$  was also observed compared to infection with WT bacteria, and this corresponded to reduced histamine levels. Together, these data indicate that *A. dhakensis* expresses *hdc* during infection which escalates virulence potential via an attenuated host immune response.

## Exploring the role of female sex hormones in host defense against nontuberculous mycobacteria

Christine R. Ponce<sup>1</sup>, Kristin T. Dean<sup>2</sup>, Rachel N. Wilsey<sup>2</sup>, Pamela J. McShane<sup>3</sup>, Jennifer R. Honda<sup>2</sup>

<sup>1</sup>Biotechnology Master of Science Program, Department of Cellular and Molecular Biology, School of Medicine, University of Texas Health Science Center at Tyler, Tyler, United States. <sup>2</sup>Department of Cellular and Molecular Biology, School of Medicine, University of Texas Health Science Center at Tyler, Tyler, United States. <sup>3</sup>Critical Care Medicine and Pulmonary Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, United States

### Abstract

**Background:** Nontuberculous mycobacterial lung disease (NTMLD) disproportionately affects post-menopausal women, suggesting a role for female sex hormones (FSexH) in disease susceptibility. We hypothesize reduced FSexH levels promote mycobacterial persistence through altered macrophage immune responses and impaired airway epithelial defenses. **Methods:** Plasma from post-menopausal bronchiectatic patients without NTM infection (n=10) and healthy pre- and post-menopausal donors (n=37) were analyzed for FSexH dehydroepiandrosterone (DHEA), estradiol (E2), progesterone (P4) and TNF- $\alpha$ , and IL-1 $\beta$  by ELISA. Monocyte derived macrophages (MDM) infected with *Mycobacteria chimaera* (MCHIM) were analyzed for changes in cell associated CFU and cytokine production. Similarly, MDM from pre- and post-menopausal healthy women infected with *Mycobacteria abscessus* (MABS) and supplemented with E2 or P4 were assessed for changes in CFU and cytokine production. Because FSexH can affect cilia movement and infection, physiologically relevant concentrations of P4 were added to apical out airway organoids (AOAOs) and organoid movement was digitally documented. **Results:** Plasma samples from bronchiectatic females exhibited significantly lower circulating E2 levels (p<0.01) and higher plasma IL-1 $\beta$  levels (p<0.05) compared to healthy donors, while DHEA, P4, and TNF- $\alpha$  levels were similar between groups. Between bronchiectasis and healthy donor groups without the addition of hormones, MCHIM was similarly controlled. Assessment of MDM response in the presence of hormones is still in progress. AOAOs were successfully generated and exhibited decrease movement when treated with P4 for 96 hours. **Conclusions:** Our findings of decreased circulating E2 levels, increased plasma IL-1 $\beta$ , and reduced organoid movement in the presence of P4 suggest FSexH may influence control of NTM.

## Optimizing Single-Cell Infection Conditions to Identify Intracellular Survival Strategies of *Paraburkholderia* in *Dictyostellium discoideum*

Sadia Islam Reya, Margaret Steele

Texas Tech University, Lubbock, United States

### Abstract

#### Abstract

*Dictyostellium discoideum* naturally feeds on bacteria through phagocytosis and is a useful model for studying intracellular host-pathogen interactions. Several *Paraburkholderia* species can survive within *D. discoideum*, but the bacterial genes required for fitness during this interaction are still unknown. Transposon sequencing (Tn-seq) uses a pooled library of bacterial mutants containing transposon-disrupted genes to determine which mutants are depleted or enriched following a selective condition that allows identification of genes required for bacterial fitness. However, co-infection of a single amoeba by multiple bacterial mutants can cause complementation, potentially rescuing mutants with disrupted fitness genes and masking their true phenotypes. So, we aimed to optimize infection conditions that maximize single infection while minimizing double infection. *D. discoideum* were infected with GFP- and RFP-labelled *Paraburkholderia* at multiplicities of infection (MOIs) of 0.3, 0.6, and 0.9 bacteria per amoeba. Infection outcomes were analyzed using fluorescence microscopy and flow cytometry. Flow cytometry showed that co-infection increased with MOI, from 0.51% at MOI 0.3 to 10.9% at MOI 0.6 and 17.3% at MOI 0.9. MOI 0.3 produced minimal co-infection but the lowest overall infection, whereas MOI 0.9 produced higher infection efficiency with increased co-infection. MOI 0.6 provided the optimal balance between infection efficiency and single infection, with clear GFP and RFP populations and moderate co-infection. These optimized conditions developed a framework for future Tn-seq experiments to identify bacterial genes contributing to fitness during interaction with *D. discoideum*.

## Prdm1/Blimp-1 acts as a corepressor of FoxA to suppress esophageal gland cell fate in schistosome parasites

Ryan Sloan<sup>1</sup>, Pallavi Yadav<sup>1</sup>, Pengyang Li<sup>2</sup>, Sabona Simbassa<sup>1</sup>, Bo Wang<sup>2</sup>, Jayhun Lee<sup>1</sup>

<sup>1</sup>UTHealth Houston, Houston, United States. <sup>2</sup>Stanford University, Stanford, United States

### Abstract

**Background:** In schistosomes, parasitic flatworms that cause the devastating disease known as schistosomiasis, the forkhead transcription factor FoxA regulates the development/maintenance of the esophageal gland (EG), an organ essential for immune evasion and survival *in vivo*. In addition to its EG expression, prior single-cell studies show *foxA* expression in a limited number of neural subsets and intestinal progenitors. However, we lack a comprehensive understanding of the cell-type-specific expression and function of FoxA. A pioneer factor such as FoxA works with cofactors to promote or repress lineage-specific gene expression by altering chromatin accessibility. Thus, we hypothesize that schistosome FoxA works with tissue-specific cofactors to regulate the production and/or function of non-EG cell types. **Methods:** To enrich for *foxA*-expressing cell types and to identify potential FoxA cofactors, we performed a head-enriched (HE) single-cell (sc) RNA-seq coupled with *in situ* hybridization (ISH) and RNAi of candidate genes. **Results:** HE scRNA-seq shows *foxA* expression in EG, stem cells, intestine, and neurons. Highly associated with *foxA* expression in these non-EG cell types is *prdm1/blimp-1* (validated with ISH), encoding a known transcriptional repressor and FoxA cofactor. RNAi-mediated *prdm1/blimp-1* knockdown significantly increased ectopic EG cell formation at the expense of the intestinal lineage, suggesting its role in suppressing the EG fate in non-EG cells. Motif analysis identified both FoxA and Prdm1/Blimp-1 motifs in the *foxA* regulatory region, suggesting this suppression may occur via negative regulation of *foxA*. **Conclusions:** We conclude that Prdm1/Blimp-1 acts as a corepressor of EG cell fate in non-EG *foxA* expressing cells.

## Analyzing virulence and antibiotic resistance in a hypervirulent clinical polymicrobial *Acinetobacter baumannii* and *Staphylococcus aureus* isolate

Nathaniel Thomas, Nicholas Dillon

The University of Texas at Dallas, Richardson, United States

### Abstract

Polymicrobial infections are often associated with increased antibiotic resistance, pathogen virulence, and length of hospital stay. This is especially relevant when the gram negative *Acinetobacter baumannii* and gram positive *Staphylococcus aureus* co-infect a patient. These microorganisms are categorized as priority pathogens and critical threats according to the WHO and CDC, and we are working to understand the interplay between antibiotic resistance and virulence in polymicrobial infections with these bacteria. We selected the laboratory strains AB5075 and TCH1516 to compare to our clinical polymicrobial isolates that were initially classified as pan-sensitive (PS). This classification was confirmed after determining both clinical isolates were susceptible to nearly every antibiotic tested. After observing mucoid growth from the PS *A. baumannii* strain, we measured biofilm formation in the different strains. We determined the PS *A. baumannii* strain formed a significant amount of biofilm while the PS *S. aureus* strain formed little to none. Surprisingly, the polymicrobial isolate also exhibited little biofilm formation. Despite limited biofilm formation, the polymicrobial isolate demonstrated significantly greater lethality when compared to the individual PS strains. Together, these findings suggest hypervirulent phenotypes of polymicrobial isolates occur independently from antibiotic resistance and biofilm formation. With additional research we hope to gain better mechanistic understandings of polymicrobial virulence between *A. baumannii* and *S. aureus*.

## The Effect of *E. coli* Conditioned Media on the Virulence Traits of Uropathogenic Co-isolated Strains of *E. faecalis*

Satya Vedula<sup>1</sup>, Carter Pruitt<sup>1</sup>, Jacob Hogins<sup>1</sup>, Philippe E. Zimmern<sup>2</sup>, Lydia Freddolino<sup>3</sup>, Lawrence Reitzer<sup>1</sup>

<sup>1</sup>University of Texas at Dallas, Richardson, United States. <sup>2</sup>University of Texas at Southwestern Medical Center, Dallas, United States. <sup>3</sup>University of Michigan Medical Campus, Ann Arbor, United States

### Abstract

Urinary Tract Infections (UTIs) are the most common community acquired bacterial infections. *Escherichia coli* is the cause of 50-75% of UTIs. However, 25% of *E. coli* dominant UTIs coisolate with *Enterococcus faecalis*. Although, *E. faecalis* is generally regarded as a commensal, we investigated if it exhibits pathogenic phenotypes *E. coli*-conditioned media by assessing the effect metabolic cross-talk has on the virulence of *E. faecalis* after exposure to *E. coli*-spent media. We hypothesize that *E. faecalis* will exhibit increased virulence traits in spent media.

To explore this interaction, *E. coli* and *E. faecalis* were grown overnight on selective media to ensure pure cultures. Next, colonies were inoculated into 1mL of rich media. After a two-hour pre-growth, the cultures were diluted to OD<sub>600</sub> 0.01 in 10mL of rich media and grown for 2.5 hours at 37C. After growth, the cultures were centrifuged, supernatants were filter-sterilized twice, and fresh *E. faecalis* preculture was added to 10mLs of the *E. coli* conditioned media. After another 2.5 growth period, the cells were tested for eukaryotic cell association and virulence using yeast-agglutination, hemolysis assays, and biofilm assays.

We found that *E. faecalis* exhibited increased agglutination and biofilm production in *E. coli* spent media compared to its monocultures. The increase in virulence when grown in *E. coli* conditioned media suggests that interactions between bacterial species shapes pathogenic behavior. Treatments that target each bacteria without accounting for their interactions could overlook the underlying mechanisms contributing to infection persistence and disease.

## Beyond Cell Division: GpsB Promotes MRSA Fitness During Host Cell Infection

Oscar Villasana Espinosa, Sarahi Hernandez, Omar Elkassih, Qing Tang

Univeristy of Texas at Arlington, Arlington, United States

### Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of antimicrobial resistance associated mortality worldwide. Although traditionally considered an extracellular pathogen, *S. aureus* can survive within host cells, providing a potential reservoir that contributes to persistence and evasion of host defenses. The bacterial factors that promote survival in the intracellular environment remain incompletely understood. We performed a genome-wide screening using Nebraska transposon library to identify genes contributing to bacterial fitness during macrophage infection. This approach identified *gpsB*, which encodes a conserved regulator of cell division and cell wall synthesis, as an important determinant of MRSA intracellular survival. We characterized the intracellular survival of both *gpsB* transposon (*gpsB*::Tn) and knockout ( $\Delta$ *gpsB*) mutants of USA300 JE2 strain using macrophage infection assays and found that loss of *gpsB* resulted in impaired bacterial fitness during macrophage infection. By monitoring *in vitro* bacterial growth under conditions that mimic the stresses encountered within macrophages, we found that  $\Delta$ *gpsB* exhibited increased susceptibility to reactive nitrogen and oxygen species (ROS and RNS) and acidic conditions, compared to JE2. Importantly, inhibition of ROS and RNS production, and phagosome acidification in macrophages rescued the intracellular growth defect of the *gpsB*::Tn mutant. Together, these results support a role for GpsB in promoting MRSA survival and adaptation within the intracellular environment. Ongoing studies are aimed at defining the mechanisms connecting GpsB-dependent cellular processes to intracellular survival.

## Early Career Fellows Posters

## A Bug's Heel: Exploring the Vulnerabilities of Antibiotic-Resistant *E. coli*

ABHISHEK ANAND, Ha Do, Cindy Escobar, Souvik Bhattacharyya  
UTHealth Science Centre at Houston, HOUSTON, United States

### Abstract

Antibiotic resistance is a global health problem. Because antibiotics target essential cellular functions, resistance mechanisms create fitness defects in bacteria that can be exploited as new therapeutic opportunities. Yet, the fitness landscape of antibiotic resistance remains poorly understood. In this study, we used  $\beta$ -lactam resistance in *E. coli* as a foundational model system and asked a simple question: which genes become essential only in  $\beta$ -lactamase-carrying strains? Using clinically relevant plasmid-borne  $\beta$ -lactamases (KPC-3, OXA-198, and L1-1), we mapped resistance dependencies using two complementary approaches. TnSeq (~1.5 million mutants) captured complete-loss phenotypes in nonessential genes, while CRISPRi (~4,000 single-gene knockdowns) extended the screen to essential, semi-essential, and dosage-sensitive genes. The validated genetic hits collectively revealed that specific functions within central carbon metabolism (*aceF*, *ackA*) and periplasmic redox homeostasis (*cydD*, *cysW*) that become essential in resistant strains. *aceF* routes pyruvate, potentially from peptidoglycan turnover, into acetyl-CoA, while *ackA* carries the downstream acetyl-phosphate-to-acetate step, generating ATP and balancing carbon between peptidoglycan synthesis and energy production. *cydD* exports cysteine/glutathione to the periplasm, while *cysW* imports sulfate/thiosulfate for cysteine synthesis, sustaining periplasmic thiol redox balance. We propose a model in which antibiotic entry and  $\beta$ -lactamase hydrolysis compete kinetically, allowing transiently free  $\beta$ -lactam to reach PBPs and impose cell-wall stress. This increases demands for peptidoglycan repair and energy production, creating dependence on *aceF* and *ackA*. Concurrently, disulfide bond-containing  $\beta$ -lactamases depend on periplasmic thiol redox balance for proper folding, creating dependence on *cydD* and *cysW*. Together, these findings reveal exploitable vulnerabilities created by  $\beta$ -lactam resistance.

## NARROW-SPECTRUM ANTIBACTERIAL THERAPEUTICS THAT SELECTIVELY TARGET *CLOSTRIDIODES DIFFICILE*

Nishtha Chandal<sup>1</sup>, Azul F Silveyra<sup>1</sup>, Chetna Dureja<sup>1</sup>, Dianqing Sun<sup>2</sup>, Kirk Hevener<sup>3</sup>, Julian Hurdle<sup>1</sup>

<sup>1</sup>Institute of Biosciences & Technology, Center for Infectious & Inflammatory Diseases, Texas A&M Health Science Center, Houston, United States. <sup>2</sup>Department of Pharmaceutical Sciences, The Daniel K. Inouye College of Pharmacy, University of Hawaii, Hawaii, United States. <sup>3</sup>Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Tennessee, United States

### Abstract

*Clostridioides difficile* infection is a leading cause of antibiotic-associated infections, morbidity, and mortality in healthcare settings. Recurrence following treatment with vancomycin or metronidazole occurs in over 20% of patients and may be driven by persistent dysbiosis of the gut microbiota. Although the narrow-spectrum antibiotic fidaxomicin is associated with a reduced risk of recurrent CDI, resistance has already emerged, highlighting the need for novel narrow-spectrum therapeutics. We previously validated the *C. difficile* enoyl ACP-reductase FabK as a drug target and demonstrated that a first-generation lead compound (296), belonging to pyridinyl-thiazole-imidazole class, exhibited narrow-spectrum activity, non-absorption from large intestine, and efficacy in mice with CDI. To advance PTIMs toward drug lead candidates, we conducted structure-activity relationship studies incorporating SAR-by-catalogue screening, chemical synthesis and expansion, enzymatic assays, and pre-clinical microbiological assessment. These efforts led to the discovery of second-generation inhibitors. Analysis of >100 molecules identified a second-generation PTIM series leading to four lead candidates with minimum inhibitory concentrations ranging from 0.1 to 1.6 µg/mL against *C. difficile* R20291. Using isogenic paired *Enterococcus faecalis* strains expressing either FabK or FabI, PTIMs selectively inhibited only the FabK-expressing strain and lacked activity against *Bacteroides* spp. Collectively, these findings position PTIMs for advanced pre-clinical *in vitro* and *in vivo* mechanistic and drug evaluation studies, including pharmacokinetic/pharmacodynamic analysis, resistance profiling, spectrum-of-activity using MIC testing against gut microbiota and murine microbiome models, and efficacy tests in animal models of CDI.

## Environmental Contamination Reshapes Microbial Community Composition but Preserves Functional Potential

Pawan Devkota, Candice Lumibao

Texas A & M University - Corpus Christi, Corpus Christi, United States

### Abstract

Environmental disturbances can reshape soil microbial communities, but whether ecosystem functional potential is maintained through functional redundancy remains poorly understood. We used shotgun metagenomic sequencing to evaluate taxonomic composition and functional potential of microbial communities across a contamination gradient in coastal dune soils on Mustang Island, Texas, including metal-contaminated, oil-contaminated, and uncontaminated sites.

Read-based taxonomic profiling and MetaCyc pathway analysis showed strong taxonomic restructuring despite relatively stable functional potential. Taxonomic alpha and beta diversity differed significantly among contamination treatments, with oil-contaminated soils exhibiting the greatest diversity, whereas functional pathway diversity and composition showed little change. Differential abundance analysis identified numerous bacterial and fungal taxa enriched in oil-contaminated sites, including *Actinobacteria*, *Sphingomonadales*, and arbuscular mycorrhizal fungi, while ammonia-oxidizing archaea were consistently depleted. In contrast, only a small number of MetaCyc pathways differed among treatments, and their fold changes were modest relative to the pronounced taxonomic shifts.

Together, these findings demonstrate that contamination reshapes microbial community composition far more strongly than functional potential, suggesting substantial functional redundancy within contaminated soil microbiomes. The maintenance of functional potential despite taxonomic turnover indicates that multiple microbial lineages contribute overlapping metabolic functions, potentially buffering ecosystem processes against contamination induced disturbance.

## Population Density Mediates a Cefiderocol Reverse Inoculum Effect via Pyochelin Signaling in *Pseudomonas aeruginosa*

Donghoon Alex Kang<sup>1</sup>, Qi Xu<sup>2</sup>, Farhanah Mustafa<sup>1</sup>, Andrew Pountain<sup>2</sup>, Rodrigo Baptista<sup>1</sup>, William Miller<sup>1,3</sup>

<sup>1</sup>Houston Methodist Research Institute, Houston, United States. <sup>2</sup>University of Texas McGovern Medical School, Houston, United States. <sup>3</sup>Weill Cornell Medical College, New York, United States

### Abstract

Cefiderocol (FDC) is a siderophore-cephalosporin reserved for extensively-drug-resistant *Pseudomonas aeruginosa*. While FDC is an inefficient substrate for drug efflux and stable against most  $\beta$ -lactamases, *P. aeruginosa* readily acquires TonB-dependent receptor mutations upon treatment, limiting drug import. These mutations primarily target the catechol uptake pathway or the ferripyochelin receptor FptA, and clinical strains with drug uptake mutations frequently exhibit heteroresistance. We recently identified a heteroresistant strain (PA3-9) with a distinct phenotype by Population Analysis Profile, exhibiting FDC resistance at low bacterial densities.

At standard inoculum, the MIC of PA3-9 was 0.03  $\mu\text{g/mL}$  with a disk diameter of 32 mm. At  $10^{-3}$  dilution, PA3-9 exhibited resistance by disk diffusion (9 mm) with a 32-fold increase in the broth microdilution MIC. This phenomenon was not dependent on the chromosomal cephalosporinase AmpC. At low inoculum, the strain was resensitized to FDC in the presence of spent media from PA3-9 or PA14, specifically the low molecular weight fraction. From a screen of PA14 mutants, we determined that the siderophore pyochelin (PCH) was required for FDC sensitization. Consistent with PCH's role in regulating the expression of its cognate receptor FptA, purified PCH suppressed the inoculum effect. During bacterial growth from a low initial density, we observed the population switch to a more FDC-susceptible profile through increased expression of *pchA* (PCH biosynthetic gene) and *fptA*. Expression of *fptA* from a non-native promoter restored FDC susceptibility at low inoculum conditions. These results demonstrated that PCH functioned as a quasi-quorum-sensing molecule to drive a novel FDC inoculum effect.

## Transcriptomic Response of *Salmonella* Montevideo to Zein-Chitosan Nanoencapsulated Curcumin and Quercetin

Myungji Kim<sup>1</sup>, Seungchan Kim<sup>2</sup>, Javad Barouei<sup>1,3</sup>, Matthew Taylor<sup>4</sup>

<sup>1</sup>College of Agriculture, Food, and Natural Resources (CAFNR) Research, Prairie View A&M University, Prairie View, United States. <sup>2</sup>Department of Electrical and Computer Engineering, Prairie View A&M University, Prairie View, United States. <sup>3</sup>Human Nutrition and Food, Prairie View A&M University, Prairie View, United States. <sup>4</sup>Department of Animal Science, Texas A&M University, College Station, United States

### Abstract

*Salmonella enterica* is a significant cause of foodborne gastrointestinal illness. Curcumin (Cm) and quercetin (Q), both plant-derived polyphenolic compounds, exhibit promising antimicrobial activity but are limited by poor aqueous solubility and bioavailability. Nanoencapsulation via zein-chitosan complexes has been employed to address these constraints. To elucidate the pathogen's transcriptomic responses underlying the antimicrobial mechanisms of encapsulated Cm and Q, *Salmonella* Montevideo at exponential phase (~6 log CFU/mL) was exposed to nanoencapsulated Cm or Q at minimum bactericidal concentration (75 µg/mL) or sterile deionized water as a control for 6 h, followed by RNA sequencing. A total of 1,555 DEGs were identified under Cm treatment (806 downregulated, 749 upregulated) and 1,271 under Q treatment (607 downregulated, 664 upregulated) at  $p_{\text{adj}} < 0.05$  and  $|\log_2\text{FC}| > 1$ , when compared to control, with 58.1% of upregulated and 68.4% of downregulated genes shared between the two treatments. Strikingly, both compounds downregulated genes broadly associated with stress response, including *katE*, which is responsible for catalase-mediated H<sub>2</sub>O<sub>2</sub> detoxification ( $\log_2\text{FC} = -5.04$  and  $-4.37$  under Cm and Q, respectively), as well as ABC transporter genes involved in amino acid, sugar, and metal ion uptake, indicating impaired oxidative damage control and nutrient acquisition. Genes governing biofilm formation, including the master regulator *csgD* ( $\log_2\text{FC} = -3.99$  and  $-4.44$ , respectively), were likewise downregulated, while flagellar motility genes were upregulated ( $\log_2\text{FC}$  range = 1.01–2.51). These results suggest that nanoencapsulated Cm and Q may be useful as natural antimicrobial agents against *Salmonella* Montevideo through inhibition of cellular oxidative stress defenses.

## Improving Antibiotic Efficacies Against Urinary Tract Pathogens Using Bicarbonate

Caroline Laughlin-Black, Nicholas Dillon, Nicole De Nisco

The University of Texas at Dallas, Richardson, United States

### Abstract

Urinary tract infections (UTIs) create a significant burden on global healthcare, affecting over 400 million people each year. These infections are further complicated by antimicrobial resistance (AMR), potentially leading to life-threatening urosepsis. With novel antibiotic discovery stalling, we must cultivate new strategies to combat resistance. One such strategy is to modulate infection environments to enhance the efficacies of existing antibiotics. For example, previous research has demonstrated that bicarbonate (a biological buffer present across body sites) influences the antimicrobial susceptibilities of pulmonary pathogens. This project evaluates bicarbonate's ability to potentiate antimicrobial activity against a variety of UTI pathogens in physiologically relevant conditions.

Antimicrobial susceptibility testing (AST) was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines in both CA-MHB and artificial urine medium (AUM) in the presence and absence of 24 mM bicarbonate using clinical isolates of multiple uropathogenic species, (uropathogenic *Escherichia coli* (UPEC), *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*). Minimum inhibitory concentrations (MIC) for multiple antibiotics were compared between bicarbonate supplemented and unsupplemented conditions for each strain. The impact of bicarbonate on MICs was further assessed in human urine.

These results demonstrate that antibiotic efficacy against UTI pathogens is potentiated by bicarbonate in CA-MHB, AUM, and human urine. Azithromycin especially displayed significantly reduced MICs in the presence of bicarbonate. This data supports the potential for bicarbonate modulation to enhance antibiotic efficacy in UTI treatment. As bicarbonate is already used clinically to combat kidney stones, bicarbonate may represent a promising and readily available adjuvant therapy to improve antibiotic efficacy against UTIs.

## Influenza A surveillance in Human Clinical Samples and Wild Birds in Galveston County

Lyudmyla V. Marushchak<sup>1</sup>, Caitlin J. Cotter<sup>2,3,4</sup>, Judith U. Oguzie<sup>5</sup>, Corri Levine<sup>1</sup>, Charles O. Cass<sup>1</sup>, Philip K. Keiser<sup>6</sup>, Thang Nguyen-Tien<sup>1</sup>, Jessica Rodriguez<sup>1</sup>, Ismaila Shittu<sup>1</sup>, Claudia M. Trujillo-Vargas<sup>1</sup>, Amanda E. Wolff<sup>6</sup>, Shivonne M. Ryans<sup>6</sup>, Robert D. Kaufman<sup>6</sup>, Jillian D. Clack<sup>2</sup>, Susan L. F. McLellan<sup>1,7</sup>, Gene G. Olinger<sup>8</sup>, Gregory C. Gray<sup>1</sup>

<sup>1</sup>Division of Infectious Diseases, Department of Medicine, University of Texas Medical Branch, Galveston, United States. <sup>2</sup>Department of Epidemiology, School of Public Health and Health Professions, University of Texas Medical Branch, Galveston, United States. <sup>3</sup>Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, United States. <sup>4</sup>Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, United States. <sup>5</sup>Division of Infectious Diseases, Department of Internal Medicine, University of Texas Medical Branch, Galveston, United States. <sup>6</sup>Galveston County Health District, Galveston, United States. <sup>7</sup>Special Pathogens Excellence in Clinical Treatment, Readiness, and Education, Galveston, Galveston, United States. <sup>8</sup>Galveston National Laboratory, University of Texas Medical Branch, Galveston, United States

### Abstract

Influenza A viruses represent a global public health and veterinary health issue. Highly pathogenic avian influenza A (H5N1) has caused substantial outbreaks in birds and has been detected in humans, underscoring the risk of zoonotic transmission. This study aimed to present the results of influenza A surveillance in human clinical specimens and the characterization of A(H5N1) viruses detected in birds in Galveston County as part of a community-based One Health surveillance program. Between November and December 2025, in Galveston County, we collected 122 human nasopharyngeal swabs from UTMB clinics and oropharyngeal and cloacal swabs from dead wild and domestic birds. Human clinical specimens were tested for Influenza A by RT-PCR using the BioFire RP2.1. There was no screening for bird exposure in the collected human samples. Specimens from birds were studied by RT-qPCR, virus isolation, and NGS. Among the 122 human clinical samples, Influenza A was detected in 23 samples (18.85%), of these 23 were identified as H3; 10 (8.20%) were positive for other respiratory viruses, while 89 samples (72.95%) were negative. Among the specimens collected from birds, 7 of 10 birds (70%) had molecular evidence of HPAI H5N1. NGS identified the viruses as clade 2.3.4.4b, consistent with genotype D1.1. This study demonstrated the importance of One Health surveillance of influenza A at the human–animal–environment interface. Conducting simultaneous surveillance for rapid detection of influenza A can support earlier detection and characterization of emerging respiratory viruses for strengthening global health security and preparedness for zoonotic threats.

## Drying medications reshape the oral microbiome: experimental and population-level evidence

Oliyad Jeilu Oumer, Weerakkody Ranasinghe, Diana M. Proctor

Department of Microbiology and Molecular Genetics, McGovern Medical School, UTHealth Houston, Houston, United States

### Abstract

Population-scale analyses of the National Health and Nutrition Examination Survey (NHANES) show that oral microbiome diversity decreases with age. We hypothesized that medication use, which also increases with age, contributes to this association. Medications are the most common cause of hyposalivation, or reduced salivary flow, which increases dental cavity risk and reduces oral microbiome diversity. To determine whether medication burden contributes to age-associated loss of diversity, we reanalyzed the NHANES oral microbiome data from 8,236 adults. Although medication burden was strongly correlated with age ( $r = 0.40$ ,  $P < 0.001$ ), it was also associated with medical frailty: 77% of highly frail adults aged 20-39 took medications expected to cause dry mouth, known as xerogenic drugs, compared with 43% of non-frail adults over the age of 80. After adjusting for age and medication count, each additional xerogenic medication was associated with loss of diversity ( $P = 0.02$ ). Greater xerogenic burden was also associated with increased relative abundance of *Streptococcus*, *Veillonella* and *Lactobacillus*. To assess the population-level relevance of these findings, we analyzed 20 years of NHANES data. During this period, the proportion of U.S. adults taking at least one prescription medication increased from 43% to 49%, while the proportion taking five or more increased from 6% to 13%, and the proportion taking xerogenic medications increased from 21% to 30%. Thus, xerogenic exposure is increasingly common, while its effects on the oral microbiome remain poorly understood. We are now investigating these effects by experimentally suppressing salivary flow in healthy humans.

**A novel formaldehyde responsive protein FlhT co-regulates methylotrophic growth with FlhSR in *Paracoccus denitrificans*.**

Trusha Parekh, Marcus Tsai, Cheyenne Ziegler, Sneha Narvekar, Faruck Morcos, Stephen Spiro  
The University of Texas at Dallas, Richardson, United States

**Abstract**

*Paracoccus denitrificans* is a model organism for studies of respiratory metabolism and methylotrophy, being able to use methanol, methylamine, formaldehyde and formate as sole sources of carbon and energy. A two-component system, FlhS-FlhR, activates the expression of the genes required for C1 metabolism. Interestingly, FlhS lacks similarity to any known transmembrane or signal sensing domains, suggesting that it may utilize a secondary sensor protein for regulon activation. FlhS and FlhR are co-expressed with a standalone FIST-domain protein, FlhT. Currently, in bacteria, the only characterized FIST-domain protein is a hemoprotein, NosP (nitric oxide sensing protein), which controls the autophosphorylation of its cognate histidine kinase in response to nitric oxide (NO). Thus, we hypothesize that, upon sensing formaldehyde, FlhT interacts with FlhS to activate its autokinase and/or phosphotransfer activities.

Strains deleted for *flhS*, *flhT* and *flhR* exhibit growth and regulatory defects on methanol, methylamine, formaldehyde, but not on formate. Assays of FlhSR-dependent promoter and enzyme activities are consistent with FlhT being required for FlhSR signaling. Evidence from direct coupling analysis and AlphaFold multimer suggests that two FlhT molecules interact with a coiled-coil in the N-terminal region of an FlhS dimer. Furthermore, FlhT is predicted to have a formaldehyde binding pocket in its C-terminal domain.

FlhT is, thus, the first NosP-like protein to have a role in the regulation of energy metabolism rather than biofilm formation. The FlhSRT system of *P. denitrificans* presents a rare opportunity to study the interaction of a histidine kinase with a separate signal sensing protein.

## **The *Escherichia coli* *carT* Gene Correlates with Urovirulence and is Involved in Carbohydrate Metabolism in Group B2**

Andrew Petter, Jacob Hogins, Adam Guffey, Larry Reitzer

The University of Texas at Dallas, Richardson, United States

### **Abstract**

*Escherichia coli* is one of the most studied organisms in the world, known for being metabolically diverse, an opportunistic and virulent pathogen, and an important commensal. It is responsible for more than 300 million infections each year and is the second most deadly bacteria in the world. One subgroup of *E. coli*, phylogenetic group B2, contains several pathogenicity associated islands (PAIs) that are highly associated with virulence. We found that one gene from a PAI, the *carT* gene, is primarily present in group B2 and is itself highly associated with pathogenicity. The *carT* gene is a *malX* homolog and putative phosphotransferase component (PTS) EIIBC. A *carT* mutant has defective growth with glucose, xylose, and glycerol as carbon sources, is non-motile, and cannot induce *lacZ*. Our results suggest that CarT functions similarly to PtsG, a PTS EIIBC component in the same family that plays a role in regulation of energy metabolism through catabolite repression and inducer exclusion. This suggests that group B2 may have distinct energy metabolism regulation and supports the idea that *E. coli* virulence may partially be explained by group-specific virulence factors.

## Immune Evasion Mechanism of KSHV K1 Fc-Receptor Protein

Vikram Thakur<sup>1</sup>, America Rodriguez<sup>1</sup>, Armando Lerma<sup>1</sup>, Mitali Bhakta<sup>2</sup>, Marilyn Idicula<sup>2</sup>, Murali Muniraju<sup>1,2</sup>

<sup>1</sup>Department of Molecular and Translational Medicine, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center El Paso, El Paso, United States. <sup>2</sup>Graduate School of Biomedical Sciences, Texas Tech University Health Sciences Center El Paso, El Paso, United States

### Abstract

Kaposi sarcoma-associated herpesvirus (KSHV) is the causal agent of Kaposi sarcoma and two types of B-cell lymphoma. No vaccines or therapeutics exist to prevent or eradicate KSHV, in part due to its ability to evade host immune defenses, making it a public health burden and creating a critical need to define how it escapes immune surveillance. Herpesviruses, including KSHV, co-evolved with their hosts, hijacking host regulatory factors to camouflage themselves from host defenses. KSHV encodes a glycoprotein, K1, that has acquired immunoglobulin-like domains with Fc receptor (FcR)-like features. Similar domains in other herpesviruses—HSV, HCMV, and VZV—act as distractor receptors for host antiviral antibodies, forming bipolar antibody bridges that sequester antiviral antibodies while inhibiting antibody-dependent cellular cytotoxicity (ADCC), but the role of K1 in Fc-mediated immune evasion by KSHV has not been examined. We hypothesized that K1 functions as a distractor receptor for host antiviral antibodies. Computational analysis revealed that K1 structurally and functionally resembles human FcγRIIIa (CD16A), the primary Fc receptor on NK cells and macrophages. We generated a panel of K1-specific antibodies and KSHV K1 knockout virus to characterize K1 Fc-receptor function. Through binding assay we demonstrate K1 binds human IgG Fc at the cell surface. Ongoing studies will use K1-knockout virus and blocking antibodies to confirm K1-dependence, characterize the Fc epitope, and assess antibody sequestration and ADCC inhibition. These findings will elucidate a previously unrecognized KSHV immune-evasion pathway and may inform antibody-based therapeutics.

## Single-Cell RNA Sequencing to Reveal Interspecies Interactions and Heterogeneity in Mixed Biofilm of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Qi Xu, Andrew Pountain

UTHealth Houston, Houston, United States

### Abstract

*Staphylococcus aureus* and *Pseudomonas aeruginosa* are major opportunistic pathogens that exhibit increasing resistance to many frontline antibiotics. Infections caused by these multidrug-resistant strains are particularly challenging to treat due to limited therapeutic options and their ability to establish biofilms. Biofilms are structured microbial communities embedded within a self-produced extracellular matrix, which promotes bacterial persistence and protects bacterial cells from antimicrobial agents and host immune defenses. Consequently, biofilm-associated infections are often chronic, recurrent, and refractory to conventional antimicrobial therapies. Meanwhile, gradients in nutrients, oxygen, and signaling molecules generate spatial heterogeneity within these biofilms, resulting in metabolically and physiologically diverse bacterial subpopulations.

To study the dynamics between these two pathogens and their individual adaptations in biofilm, we apply single-cell RNA sequencing (scRNA-seq) to their mono- and co-culture biofilms. This approach enables high-resolution profiling of gene expression heterogeneity within biofilms and reveals how individual bacterial cells communicate within the community and interact across species. By comparing transcriptomic profiles between mono- and co-cultures, we aim to identify specific subpopulations that contribute to interspecies interactions and biofilm persistence, and better understand the role of interspecies interactions in biofilm-associated infections.

## Researchers and Faculty Posters

Session A Res P43 (Board 43)

### The CarSR Two-Component System Senses and Regulates Bacterial Aggregation in *Fusobacterium nucleatum*

Abu Amar Al Mamun, Bibek GC, Chenggang Wu  
UT Health at Houston, Houston, United States

#### Abstract

The oral pathobiont *Fusobacterium nucleatum* uses the **CarSR two-component system (TCS)** to rapidly adapt to environmental changes and to regulate coaggregation with Gram-positive bacteria. While past RNA-Seq data linked this system to the expression of the surface adhesin RadD, the specific mechanisms of the broader CarR regulon and the exact signals that trigger it remained undetermined. Using ChIP-seq, this study mapped **161 CarR-enriched loci** across the genome and identified a 17-bp consensus binding motif. This motif was located directly in the promoter region of the *radABCD* operon. The direct interaction between the response regulator CarR and this sequence was validated via EMSA, mutagenesis, and DNase I footprinting. Beyond controlling biofilm coaggregation factors, CarR acts as a global regulator that directly governs the expression of genes involved in the utilization of fructose and amino acids (cysteine, glutamate, and lysine). Finally, the study established a feedback loop where **RadD-mediated coaggregation upregulates *radD* expression**, an effect that is completely lost when *carS* is deleted. This indicates that the physical act of coaggregation serves as the primary environmental cue sensed by the CarSR system. These findings reveal a novel mechanism for how *F. nucleatum* coordinates dental plaque formation and drives polymicrobial disease processes.

## Functional and Culturomics Based Analysis of the Ocular Surface Microbiome

Alfredo Calderon-Macedo<sup>1</sup>, Taylor S. Robinson<sup>1</sup>, Jose O. Cantu<sup>1</sup>, Emavieve R. Coles<sup>1</sup>, Claire E. Bason<sup>1</sup>, Samantha G. Venables<sup>1</sup>, Karolina E. Szenkiel<sup>1</sup>, Eric L. Brown<sup>2</sup>, Kristi L. Hoffman<sup>1</sup>, Stephen C. Pflugfelder<sup>1</sup>, Cintia S. de Paiva<sup>1</sup>, Robert A. Britton<sup>1</sup>, Eva Priesner<sup>1</sup>, Laura Schaefer<sup>1</sup>

<sup>1</sup>Baylor College of Medicine, Houston, United States. <sup>2</sup>University of Texas Health Science Center, Houston, United States

### Abstract

This study focuses on cultivating and characterizing microbes from the eye, an understudied niche of the human microbiome. 100 healthy subjects with equal sex and age ratios were clinically evaluated to exclude dry eye. After informed consent, conjunctival, lid margin and glabellar swabs were collected. Tears were collected using a capillary tube. A subset of patients was recalled for longitudinal follow-up sampling after 4, 8, and 12 months post-initial visit to assess microbiome stability. Samples were cultured aerobically on the day of collection on 4 different media. Isolates were picked after plating over 7 days, cryogenically preserved, and sequenced using PCR-amplified 16S rRNA gene fragments for taxonomic identification.

To date, over 20,000 bacterial isolates have been saved in our biobank. Conjunctiva and tears had the lowest biomass with 50% of subjects yielding <11 and <44 colony-forming units, respectively. Across all sites, the most prevalent cultured genera were *Staphylococcus* and *Corynebacterium*, with conjunctiva and tears showing the least diversity. For functional analyses and characterization, we have curated a list of representative isolates from tears and conjunctiva spanning 6 genera and 19 species. SNP analysis of whole-genome sequences revealed that specific *Corynebacterium* and *Staphylococcus* strains persist across time in a subset of longitudinal patients. To assess antibiotic susceptibility profiles, we measured minimum inhibitory concentration values for 11 antibiotics commonly used for ophthalmic purposes. Finally, supernatants and heat-killed cells from these isolates are being incubated with human corneal primary explant cultures, and inflammatory cytokine expression changes are quantified by RT-qPCR.

## FtsZ forms biomolecular condensates in a polar-growing Alphaproteobacterium

Todd Cameron<sup>1</sup>, Miguel Robles-Ramos<sup>2</sup>, Lorenzo Suigo<sup>1</sup>, Silvia Zorrilla<sup>2</sup>, William Margolin<sup>1</sup>

<sup>1</sup>University of Texas Health Science Center at Houston, Houston, United States. <sup>2</sup>Centro de Investigaciones Biológicas Margarita Salas, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain

### Abstract

The bacterial cell division protein FtsZ assembles into GTP-dependent protofilaments that organize the bacterial divisome in most bacterial species. Previous work with purified *E. coli* FtsZ (FtsZ<sub>Ec</sub>) has demonstrated that FtsZ<sub>Ec</sub> can form phase-separated biomolecular condensates in vitro, possibly as a regulatory mechanism invoked during bacterial quiescence. However, no FtsZ<sub>Ec</sub> condensates have been confirmed in vivo. In contrast with FtsZ<sub>Ec</sub>, FtsZ of the Alphaproteobacterium *Agrobacterium tumefaciens* (FtsZ<sub>At</sub>) localizes to distinct polar foci during the unipolar phase of its normal growth cycle prior to cell division, and contains an unusually long intrinsically disordered region (IDR). These features suggest that FtsZ<sub>At</sub> potentially forms condensates during routine growth and its expanded IDR may increase the tendency of FtsZ<sub>At</sub> to phase-separate. We therefore investigated the condensate-forming properties of alphaproteobacterial FtsZ proteins in vivo and in vitro. Under in vitro molecular crowding conditions, we found that FtsZ<sub>At</sub> exhibited a strong tendency to form condensates, and that addition of GTP induced the formation of filaments radiating from the FtsZ<sub>At</sub> droplets. In vivo observations and FRAP analysis revealed similar FtsZ<sub>At</sub> foci that dynamically assembled and coalesced inside cells. Additionally, interconversion between FtsZ<sub>At</sub> filaments and foci could be induced by modulating the extracellular environment, suggesting that the FtsZ<sub>At</sub> phase transition may be regulated by environmental conditions. Overall, these results suggest that FtsZ<sub>At</sub> forms condensates at one pole of *A. tumefaciens* cells, perhaps to sequester FtsZ<sub>At</sub> before cell division as an adaptation to slower generation times in this bacterial species.

## Engineered a calprotectin-responsive probiotic to mitigate *Clostridioides difficile* infection

Duolong Zhu<sup>1</sup>, Yu Zeng<sup>2</sup>, Zhilei Chen<sup>2</sup>, Robert Britton<sup>1</sup>

<sup>1</sup>Baylor College of Medicine, Houston, United States. <sup>2</sup>Texas A&M University Health Science Center, Bryan, United States

### Abstract

**Introduction:** *Clostridioides difficile* infection (CDI) is a major driver of intestinal inflammation, morbidity, mortality, and healthcare burden. Although probiotics such as *Escherichia coli* Nissle 1917 (EcN) exhibit anti-inflammatory potential, existing probiotics and engineered microbes largely rely on passive supplementation or static drug delivery, resulting in modest and inconsistent clinical efficacy. The development of microbial-based precision therapies capable of responding dynamically to disease severity with real-time, site-specific drug delivery is a critical unmet need. Fecal calprotectin is a clinically validated biomarker of intestinal inflammation that reflects local gut pathology, providing an opportunity to develop precision, inflammation-responsive microbial therapies for CDI.

**Hypothesis:** *C. difficile* toxin B (TcdB) binding proteins (sT10-2h and D16) and mini-receptor miniCSPG4 can mitigate TcdB mediated pathology. Engineering EcN to sense calprotectin and respond by producing TcdB binding proteins will enable precise, localized, and timely therapeutic delivery during active inflammation.

**Methods:** Using synthetic biology, we engineered EcN that detects calprotectin and responds by releasing therapeutics via either secretion or programmed autolysis. Meanwhile, the engineered EcN was evaluated for therapeutic efficacy *in vitro* and *in vivo*.

**Results:** Engineered calprotectin-responsive EcN provides robust protection against TcdB mediated pathology both *in vitro* and *in vivo*, significantly reducing disease severity in a CDI mouse model.

**Conclusions:** Our study establishes a biomarker-responsive microbial therapeutic platform capable of site-specific delivery of therapeutic agents during CDI. This strategy overcomes the limitations of passive probiotic supplementation and introduces a modular, adaptive, and disease-responsive approach for treating CDI with a potential to improve efficacy and reduce systemic toxicity.

## Surface sensing and metabolic signals drive feedback regulation of long polar fimbriae in enterohemorrhagic *Escherichia coli* O157

Soumita Dutta<sup>1</sup>, Walter Galdamez<sup>2,1</sup>, Manuel Ramirez<sup>2,1</sup>, Anne-Marie Krachler<sup>2,1</sup>

<sup>1</sup>Department of Microbiology and Molecular Genetics, UTHealth, Houston, Houston, United States.

<sup>2</sup>Microbiology and Infectious Disease Program, MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, University of Texas, Houston, Houston, United States

### Abstract

Bacterial colonization requires a carefully coordinated response to environmental changes. While we know a lot about how metabolism regulates adhesion structures, we still don't fully understand how surface sensing fits into the picture. Here, we investigated the regulation and function of long polar fimbriae (Lpf) in enterohemorrhagic *Escherichia coli* O157. We found that the *lpf1* and *lpf2* promoters combine metabolic and surface-associated signals through distinct regulatory regions. Bile induces both promoters independently of distal regulatory sequences. In contrast, these distal regions repress baseline transcription and are necessary to turn on expression once the bacteria attach to a surface. Interestingly, this surface-dependent activation drops in mutants lacking the major fimbrial subunits LpfA1 and LpfA2, but remains unaffected when the distal tip adhesins are deleted. Lpf structures also boost the surface-dependent activation of *ler*—a positive regulator of *lpf* expression—which points to a regulatory feedback loop linking surface sensing directly to *lpf* transcription. This distinction between the fimbrial shaft and the adhesive tip also matters during infection: losing the major fimbrial subunits impairs colonization in zebrafish, but deleting the tip adhesins does not under our testing conditions. Together, our findings show that Lpf integrate environmental cues through surface-dependent regulatory feedback, revealing a novel role for the fimbrial structure during colonization that does not depend on its tip adhesins.

## Codon usage Bias and Evolutionary Determinants of Codon Preference in *Campylobacter jejuni*

Samira Alam, Syed Hasib Akhter Faruqui, [Anand B. Karki](#)

Sam Houston State University, Huntsville, United States

### Abstract

*Campylobacter jejuni* is a major foodborne bacterial pathogen whose genome exhibits substantial diversity between core and accessory gene sets. This study aimed to characterize codon usage patterns in core and accessory genes and evaluate the genomic and evolutionary factors influencing codon preference, including guanine-cytosine (GC) composition, mutational pressure, and natural selection. Out of the 113,530 *C. jejuni* genomes available in the NCBI database; 659 complete genome sequences were selected for analysis and were annotated using Bakta. Comparative pangenome analysis was performed to classify genes into core, shell, and cloud categories. Codon usage was evaluated using relative synonymous codon usage (RSCU), effective number of codons (ENC), codon adaptation index (CAI), tRNA adaptation index (tAI), and GC content. RSCU analysis revealed marked preferences among synonymous codons, with AGA being highly preferred and CGG being infrequently used for arginine. A mean CAI of 0.67 and a mean ENC of approximately 39 indicated moderate codon usage bias, whereas the relatively low mean tAI of 0.36 suggested weak adaptation to the cellular tRNA pool. CAI was inversely associated with GC content, and most genes clustered around an ENC of approximately 39 at relatively low GC3 values, supporting an association between nucleotide composition and codon usage bias. Furthermore, neutrality plot analysis suggested a stronger influence of natural selection on codon usage bias than mutational pressure (regression slope is 0.198). Overall, these findings indicate the combined effects of nucleotide composition and evolutionary effect, with natural selection being predominant force shapes the codon preference of *C. jejuni*.

## TraH Localizes at the Tip of the Conjugative F Pilus

Pratick Khara, Yang Grace Li, Peter J. Christie

McGovern Medical School, UTHealth at Houston, Texas, Houston, United States

### Abstract

Bacterial conjugation is a major mechanism responsible for horizontal gene transfer and the spread of antibiotic resistance and other adaptive traits. In Gram-negative bacteria, conjugative type IV secretion systems (T4SSs) assemble extracellular pili that mediate donor–recipient contacts required for efficient DNA transfer. In several well characterized T4SSs, members of the VirB5 family protein localize at the tip of conjugative pili and play essential roles in pilus assembly and recipient-cell recognition. Recent high-resolution structures of the F-plasmid encoded T4SS (T4SS<sub>F</sub>) and F pilus have provided unprecedented insights into their architectures, but the composition of the F pilus tip has not been defined. Here, we determined that the F-plasmid-specific protein TraH shares structural similarities with the VirB5 family of proteins, as predicted by Alphafold modeling. An N-terminal Strep-TraH variant was stable and fully functional in supporting F plasmid transfer and F pilus biogenesis. We enriched Strep-TraH from F plasmid-carrying *Escherichia coli* cells, and in the pull-downs we detected intact F pili by electron microscopy, as well as the VirB2-like pilin subunit TraA, VirB6-like TraG and, less prominently, other T4SS<sub>F</sub> machine components, by mass spectrometry. We also visualized Strep-TraH at the tip of F pili by immunofluorescence microscopy and a proximity ligation assay (PLA). Together, our findings strongly indicate that TraH functions as a VirB5-like family member that localizes at the tip of the F pilus and plays a critical role in pilus biogenesis, establishment of productive target cell interactions, and dissemination of F plasmids.

## Overcoming BamD essentiality by mutations in a non-essential substrate

Santosh Kumar, Anna Konovalova

Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, United States

### Abstract

A defining feature of Gram-negative bacteria is the outer membrane (OM), a selective permeability barrier and major contributor to intrinsic antibiotic resistance. OM  $\beta$ -barrel proteins (OMPs) play crucial roles in nutrient transport, signaling, adhesion, and virulence. OMPs are inserted into the OM by the  $\beta$ -barrel assembly machinery (Bam). The Bam complex has emerged as a major target for antibiotic development due to its essential role in OM biogenesis. However, limited understanding of Bam function remains a barrier for rational antibiotic design.

The Bam complex consists of BamA and four associated lipoproteins, BamB-E. BamA and BamD are essential, whereas BamB,C,E are individually dispensable but collectively plays essential role in regulating BamA-D core. We previously found that BamE specifically facilitates assembly of OMP-dependent, surface-exposed lipoproteins such as RcsF (a regulator of capsule synthesis protein F). In the absence of BamE, RcsF stalled on BamA, blocking its function. This is the first report that lipoproteins can block Bam complex activity and provide alternative routes for Bam inhibition.

We found that BamD essentiality due to RcsF jamming on BamA. BamE suppressors of both BamA and RcsF not only survived BamD depletion but also restored OMP assembly. However, in the absence of BamB, the effects of BamA and RcsF suppressors were nullified. Furthermore, BamD could be deleted in the RcsF suppressor (<sup>G117R</sup>) background. Our results demonstrate that BamD does not play a catalytic role in OMP assembly. Instead, BamD regulates BamA in wild-type cells, enabling its active engagement with upcoming unfolded OMP substrates.

## Long-read tagmentation unlocks scalable, cost-effective, genome-resolved metagenomics from low-input DNA in the built environment

Jeremiah Minich, Tatiana McGarry, Raghav Kataria

Baylor University, Waco, United States

### Abstract

Long-read metagenomic sequencing with PacBio HiFi remains costly and requires large amounts ( $\mu\text{g}$ ) of DNA thus limiting the applications to only a few sample types like soil, feces, and filtered water. Here, we present a long-read tagmentation and amplification based library preparation approach, LongPlex (LP), which reduces per sample library preparation costs by 76-87% and DNA input requirements (from 1,000 ng to  $\sim 10$ 's ng). We created an ESKAPE pathogen mock community consisting of five organisms to compare standard PCR-free libraries vs. tagmentation-PCR libraries under various conditions (three long range mastermixes; DNA input: 15-30, 75-150 ng, and 150-300 ng; and three LongPlex reaction volumes). Genome quality did not differ between PCR-free and PCR approaches with varying reaction volumes and DNA input, although HQ-MAG and cMAG quantities and assembly contiguity, especially in GC-rich species was lower with DeCodiFi and NEB whereas LongPlex with Takara LongPlex did not differ from PCR-free on any metric. We apply this approach to a challenging and understudied sample type (with long reads), the indoor built environment. We recovered 234 complete MAGs (51% putatively novel species) across 54 shower drain samples from nine university dorms with as little as 1 ng of DNA and 0.5 Gbp of HiFi data yielding at least one complete MAG. Antibiotic resistance genes (ARGs) were present in 24.4% of classified species. *Mycobacterium phocaicum* contained the highest cumulative number ARGs by copy likely due to higher overall abundance and distribution across drains, whereas *Stenotrophomonas maltophilia* had the highest number of ARGs per genome.

## The Sickle Cell Disease Mouse Gut Microbiome Contributes to Chronic Pain that is Reversed by the Probiotic *Akkermansia muciniphila*

Mackenzie Simms<sup>1</sup>, Amanda Brandow<sup>2</sup>, Samantha Atkinson<sup>3</sup>, Zulmary Manjarres<sup>1</sup>, McKenna Pratt<sup>1</sup>, Iti Mehta<sup>1</sup>, Sruthi Mudunuri<sup>1</sup>, Aishwarya Kappagantu<sup>1</sup>, Sahar Alhendi<sup>1</sup>, Anagha Sheshadri<sup>1</sup>, Kelli Palmer<sup>1</sup>, Kate Sadler<sup>1</sup>

<sup>1</sup>University of Texas at Dallas, Richardson, United States. <sup>2</sup>Medical College of Wisconsin, Milwaukee, Milwaukee, United States. <sup>3</sup>Medical College of Wisconsin,, Milwaukee, United States

### Abstract

Pain is the primary reason individuals with sickle cell disease (SCD), the most prevalent genetic blood disorder in the world, seek medical care. Despite this, the etiology of this pain remains poorly understood, with only a few non-opioid-based treatment options. Recent 16S rRNA gene sequencing from SCD patients revealed associated gut dysbiosis; however, it remains unclear how these microbial changes affect chronic pain. In this study, we used the Townes mouse model to assess SCD gut microbiota-associated pain. We performed fecal material transplant (FMT) from Townes SCD mice into healthy controls and found that SCD microbiota induced mechanical allodynia, cold hypersensitivity, and mechanical hyperalgesia mirroring naive SCD mice. Conversely, FMTs from healthy controls into SCD mice produced a transient partial reversal in their mechanical allodynia and cold hypersensitivity, suggesting the healthy control microbiota provides analgesia. This was supported by 16S ribosomal RNA sequencing of Townes sickle cell and healthy hemoglobin fecal samples, showing differences in several genera. Reflecting the human 16S data, bacteria from the genus *Akkermansia* were less abundant in Townes SCD fecal samples. We cultured *Akkermansia muciniphila*, an anaerobic Gram-negative bacterium, because studies have shown beneficial effects on gut epithelial integrity and production of anti-inflammatory short-chain fatty acids (SCFAs). We administered this probiotic to SCD mice and reversed their chronic pain. *A. muciniphila* reversed mechanical allodynia, cold hypersensitivity, and mechanical hyperalgesia seen in SCD mice. Our findings show a mechanistic link between the SCD gut microbiome and chronic pain, contributing to an ongoing effort to identify potential analgesics.

## The role of a type VII secretion system in *Streptococcus gallolyticus* subsp. *gallolyticus* biofilm formation

Jyoti Tamang, Sarah Woodfin, Yi Xu

Texas A&M IBT, Houston, United States

### Abstract

*Streptococcus gallolyticus* subspecies *gallolyticus* (*Sgg*) is a gut pathobiont that is strongly associated with colorectal cancer. It also causes infective endocarditis (IE), accounting for approximately 15% of all IE cases worldwide. Biofilm formation is a significant virulence trait, contributing to disease severity and antibiotic treatment failure. The bacterial Type VII Secretion System (T7SS) is a specialized secretion system that primarily mediates host-pathogen interactions and interbacterial competition. Our recent studies based on genomic comparison and correlation analysis suggested that T7SS may play a role in *Sgg* biofilm formation (Calderon *et al.*, Front Microbiol., 2026). We investigated this further. A panel of deletion mutants lacking specific T7SS components were analyzed. We found that some mutants displayed reduced biofilm formation ability while others did. Interestingly, the latter group included deletions of T7SS core components that are believed to be essential for a functional secretion apparatus. Transcriptomic and proteomic analysis revealed that mutants in the former group exhibited significant downregulation of genes in the pilus 3 (*pil3*) locus. Previous studies have demonstrated that Pil3 is involved in biofilm formation in *Sgg* in response to cyclic di-AMP levels. Together, these findings suggest a novel mechanism in which T7SS mediates biofilm formation by regulating Pil3. Studies are on-going to further delineate the role of T7SS in *Sgg* biofilm formation.

## Therapeutic Effectiveness of EntV P4D Peptide Against Fungal Infections

Luis Alberto Vega, Giuseppe Buda De Cesare, Melissa Cruz, Michael Lorenz, Danielle Garsin  
McGovern Medical School, UTHealth Houston, Houston, United States

### Abstract

Fungal infections are estimated to kill 1.5-3.5 million people annually. Presently, only three broad classes of antifungals are therapeutically available (polyenes, azoles, echinocandins). Antifungal-resistant isolates of various species (e.g. *Candidozyma auris*, *Candida albicans*, *krusei*, *parapsilosis*, *tropicalis*, *Nakaseomyces glabrata*) are increasingly associated with life-threatening disease, particularly among immunocompromised patients. Widespread resistance to existing antifungals is of great public health concern, particularly given the paucity in development of novel antifungal classes. Our labs have developed a 10 residue D-amino acid variant of the EntV peptide produced by *Enterococcus faecalis*, which inhibits *Candida albicans* pathogenicity, referred to as P4D (patent# PCT/US2023/065708). P4D not only retains the full anti-infective capability of the active form of EntV, but exhibits enhanced stability under physiological conditions (e.g. in serum). In this study we have preliminarily tested the therapeutic capability of P4D in mouse models of systemic candidiasis. In these models, orally and intravenously delivered P4D showed significant efficacy in reducing infectious fungal burden of multiple species in either immunocompetent (e.g. *C. albicans*, *N. glabrata*) or immunocompromised hosts (*C. auris*). Analysis of immunological responses associated with P4D revealed a significant increase in pro-inflammatory cytokines levels in plasma (i.e. IL-1 $\alpha$ , IFN- $\gamma$ , TNF- $\alpha$ , MCP-1, IL-10, IL-6) in mice with systemic candidiasis. While preliminary testing revealed no synergy with azole or echinocandin treatment, future work will further test P4D anti-infective potential against other fungal genera and anti-fungal resistant isolates and define the mechanisms by which P4D both inhibits fungal virulence and promotes host immunity.

## KSHV Spatial Metabolomic and Lipidomic Pilot Study

Geetanjali Venna<sup>1</sup>, Fatima Hisam<sup>1</sup>, Eranda Berisha<sup>1</sup>, Erin Seeley<sup>2</sup>, Erica Sanchez<sup>1</sup>

<sup>1</sup>The University of Texas at Dallas, Richardson, United States. <sup>2</sup>MD Anderson, Houston, United States

### Abstract

Kaposi's Sarcoma Associated Herpesvirus (KSHV) is an oncogenic gammaherpesvirus that causes Kaposi's Sarcoma (KS). The reddish-purple lesions characteristic of KS are composed of infected endothelial cells. A majority of infected cells in KS lesions exist in the latent stage of KSHV infection with a smaller population in the lytic stage. During latency, the host cell survives and the KSHV episome persists in the nucleus with expression of only a few viral genes. Following reactivation, KSHV lytic infection leads to expression of all viral genes and infectious virion production. Our targeted bulk metabolomic and lipidomic studies on KSHV lytic infection have shown that KSHV majorly depends on host metabolic pathways during infection. Host nucleotide metabolism such as purine, pyrimidine, as well as phospholipid and neutral lipid metabolism are altered during lytic KSHV infection when compared to latent infection. However, these bulk studies do not account for the endothelial heterogeneous subpopulation of cells in different viral stages of infection as well as the uninfected bystander cells. Hence, we have proposed a spatial metabolomic and lipidomic study of KSHV infection using the iSLK.BAC16 reporter cell line which includes lytic, latent and un-infected control samples. These samples are pelleted and sectioned for detection of metabolites and lipids using MALDI-MSI. This study is a novel method of mapping the distribution of metabolites and lipids during various stages of KSHV infection. Defining the spatial metabolomic and lipidomic profiles of each infection stage can help identify potential antiviral therapeutic targets.

## **Growth and Extinction Dynamics of Microbial Communities Under Long-term Starvation Across Variable Nutrient Environments**

Ashley Vu, Sylvie Estrela

Texas A&M University, College Station, United States

### **Abstract**

Microbial communities experience episodic nutrient scarcity driven by natural resource fluctuation and competition. While nutrient availability is known to shape the assembly of microbial communities, how long-term starvation interacts with substrate composition, quantity, and oxygen availability to shape communities remains understudied. To investigate this, inoculated a soil-derived microbial community into minimal media supplemented with single and mixed carbon sources. We serially passaged the cultures into fresh media weekly to model feast-famine dynamics. We measured optical density (OD) and colony-forming units (CFU) to track changes in growth and death dynamics. Our preliminary results suggest that growth and extinction dynamics are influenced by the nutritional environment, and that even replicate cultures sharing the same inoculum and media showed high variability in growth and extinction outcomes. Together, these results suggest a role for both stochasticity and nutrients in shaping community dynamics. Further work will elucidate the mechanisms that drive a community to collapse or adapt under starvation.

## **A Modular Genome Engineering Platform for Conditional Depletion and Replacement of G3BP1**

Zhaohan Xu, Jared Bard

Texas A&M, College Station, United States

### **Abstract**

Chikungunya virus (CHIKV) and other Old World alphaviruses depend on the host RNA-binding proteins G3BP1 and G3BP2 for efficient replication through direct interactions with viral nsP3. However, loss of both G3BP paralogs strongly impairs CHIKV replication, making it difficult to determine which stage of infection requires G3BP and which region or interactions of G3BP support viral replication.

To address this, we developed a modular genome-engineering platform that combines acute depletion of endogenous G3BP1 with Tet-on controlled expression of mutant G3BP1 variants. A single copy Bxb1 landing pad was installed at the AAVS1 safe harbor locus, allowing us to generate highly homogeneous stable cell lines within 7 days. Endogenous G3BP1 was tagged at its C-terminus with FKBP12(F36V) for rapid depletion using the dTAG system, while G3BP2 was knocked out using a dual-guide CRISPR-Cas9 strategy to remove paralog redundancy. dTAGV1 treatment reduced tagged G3BP1 by more than 95% within 4 h in HEK293T cells and 2 h in U2OS cells. Unexpectedly, tagged G3BP1 failed to support CHIKV replication even before its degradation, producing viral E1 RNA levels similar to G3BP1/2 double knockout cells. This suggests that the C-terminal fusion disrupts G3BP2 function and the C-terminus of G3BP1 is important for CHIKV replication.

Future work will focus on redesigning the fusion configuration to preserve G3BP1 function, including test alternative epitope tag and different fluorescent protein markers. Once the function is restored, this platform will be used to introduce a G3BP1 mutant library and systematically identify regions and interactions required for CHIKV replication.

## Schistosome esophageal gland factor MEG-8.2 drives host cell lysis and interacts with host immune proteins

Pallavi Yadav<sup>1</sup>, Sabona B Simbassa<sup>2,3</sup>, Ryan Sloan<sup>4,3</sup>, Phillip A. Newmark<sup>5</sup>, Jayhun Lee<sup>1,6</sup>

<sup>1</sup>Department of Microbiology and Molecular Genetics, McGovern Medical School, The University of Texas Health Science Center at Houston, HOUSTON, United States. <sup>2</sup>Microbiology and Infectious Diseases Program, The University of Texas MD Anderson Cancer Center UTHealth Houston, Houston, United States. <sup>3</sup>Graduate School of Biomedical Sciences, Houston, United States. <sup>4</sup>Microbiology and Infectious Diseases Program, The University of Texas MD Anderson Cancer Center UTHealth Houston, HOUSTON, United States. <sup>5</sup>Howard Hughes Medical Institute, Morgridge Institute for Research, Department of Integrative Biology, University of Wisconsin–Madison, Madison, United States.

<sup>6</sup>Microbiology and Infectious Diseases Program, The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, HOUSTON, United States

### Abstract

Schistosomes are blood flukes that ingest large amounts of host blood during their intra-mammalian stage. The ingested blood contains leukocytes that can be harmful, yet the parasites survive inside the host for decades, reflecting superb immune evasion mechanisms that remain poorly understood. Our previous work discovered that FoxA, a forkhead transcription factor, drives the production of the esophageal gland, an anterior digestive organ essential for degrading ingested leukocytes and for *in vivo* survival. However, a comprehensive molecular makeup of the esophageal gland remains unclear. Importantly, the esophageal gland factors responsible for degrading ingested leukocytes, their mechanisms of action, and how such a function relates to parasite survival and immune evasion remain unknown. Here, we identify additional esophageal gland genes by taking a comparative transcriptomics approach to identify transcripts altered in *foxA* knockdown adult schistosomes. A targeted RNAi screen coupled with biochemistry reveals that specific domains of the micro-exon gene MEG-8.2, can drive host cell lysis in a concentration-dependent manner. Using pull-down assays coupled with mass spectrometry, we discover that MEG-8.2 interacts with several host membrane and extracellular proteins that play important roles in activating innate and/or adaptive immunity. Together, our findings suggest a dual role for MEG-8.2 in effectively lysing the ingested cells in the esophageal lumen and interacting with specific host proteins to neutralize or suppress host immunity. These findings lay an important foundation for exploiting esophageal gland factors to treat schistosomiasis.

## **Polo-like kinase phosphorylation of the orphan kinesin KIN-G negatively regulates flagellar inheritance in *Trypanosoma brucei***

Qing Zhou<sup>1</sup>, Yasuhiro Kurasawa<sup>1</sup>, Kyu Joon Lee<sup>2</sup>, Huiqing Hu<sup>1</sup>, Ziyin Li<sup>1</sup>

<sup>1</sup>Department of Microbiology and Molecular Genetics, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, United States. <sup>2</sup>Department of Tropical Medicine, College of Medicine, Soon Chun Hyang University, Asan, Korea, Republic of

### **Abstract**

The unicellular parasite *Trypanosoma brucei* assembles a motile flagellum that is required for locomotion, cell division plane placement, and cell-cell communication. Inheritance of the flagellum during the cell cycle relies on the faithful duplication/segregation of multiple flagellum-associated cytoskeletal structures, including a centrin-marked, bar-shaped structure termed centrin arm, which also determines the site for Golgi assembly. Biogenesis of the centrin arm requires the Polo-like kinase homolog TbPLK and the orphan kinesin KIN-G, but the mechanistic role of TbPLK in centrin arm biogenesis remains elusive. Here we report that TbPLK phosphorylates KIN-G, disrupts its microtubule-binding activity, and negatively regulates its function. TbPLK phosphorylates KIN-G *in vitro* at multiple residues, two of which are *in vivo* TbPLK phosphosites, including the Thr301 residue within one of the microtubule-binding motifs of the kinesin motor domain. Phosphorylation of Thr301 by TbPLK inhibits the microtubule-binding activity of KIN-G *in vitro*, and expression of a Thr301 phospho-mimic mutant in *T. brucei* disrupts centrin arm integrity, Golgi duplication, flagellum attachment zone elongation, flagellum positioning, and cell division plane placement. In wild-type *T. brucei* cells, Thr301 phosphorylation occurs on a small portion of the KIN-G population, suggesting that KIN-G undergoes phosphorylation/dephosphorylation cycles to regulate its activity. Together, these findings uncover a negative role of TbPLK-mediated phosphorylation of KIN-G in regulating flagellar inheritance in trypanosomes.

# Presenter Index

Alphabetical by presenter surname. Poster codes and board numbers follow the assignments in this abstract book.

[Morenikeji Adedayo](#) | Session A - GRAD P24 (Board 24) | p. 103  
[Adewale Adewole](#) | Session A - GRAD P12 (Board 12) | p. 68  
[Erum Ahmed](#) | Session A - UG P1 (Board 1) | p. 35  
[Junyoung Ahn](#) | Session A - UG P2 (Board 2) | p. 36  
[Fahadul Alam](#) | Session A - GRAD P16 (Board 16) | p. 81  
[Ahmad Ali](#) | Session A - GRAD P31 (Board 31) | p. 124  
[Nimna Amarasinghe](#) | Session A - GRAD P32 (Board 32) | p. 125  
[ABHISHEK ANAND](#) | Session A - PD P40 (Board 40) | p. 152  
[Dakota Archambault](#) | Oral presentation | p. 17  
[Ravali Arugonda](#) | Session A - GRAD P33 (Board 33) | p. 126  
[EMMANUELLA ASIAMAHA](#) | Session A - GRAD P14 (Board 14) | p. 70  
[Hossaena Ayele](#) | Session A - GRAD P34 (Board 34) | p. 127  
[Katy Ayers](#) | Session B - GRAD P30 (Board 30) | p. 128  
[Navonil Banerjee](#) | Oral presentation | p. 26  
[Eranda Berisha](#) | Session A - GRAD P27 (Board 27) | p. 112  
[Madhvi Bhakta](#) | Session A - GRAD P25 (Board 25) | p. 104  
[Umair Bhat](#) | Session A - UG P5 (Board 5) | p. 46  
[Laurel Boatright](#) | Session B - GRAD P31 (Board 31) | p. 129  
[Oliver A. Bocanegra](#) | Oral presentation | p. 11  
[Namrata Bonne](#) | Oral presentation | p. 13  
[Jennifer Bracewell](#) | Session A - GRAD P17 (Board 17) | p. 82  
[Holly Branthoover](#) | Oral presentation | p. 19  
[Guadalupe Calderon](#) | Oral presentation | p. 6  
[Alfredo Calderon-Macedo](#) | Session A Res P44 (Board 44) | p. 165  
[Todd Cameron](#) | Session A Res P45 (Board 45) | p. 166  
[John Camp](#) | Session A - GRAD P26 (Board 26) | p. 105  
[Marbella Canaca](#) | Session A - GRAD P13 (Board 13) | p. 69  
[Samuel Cannon](#) | Session B - GRAD P23 (Board 23) | p. 106  
[William Carpenter](#) | Session B - GRAD P32 (Board 32) | p. 130  
[Adriana Carrizales](#) | Session A - UG P3 (Board 3) | p. 37  
[Maria del Carmen Chacon Castro](#) | Session A - GRAD P29 (Board 29) | p. 114  
[Sarai Castro](#) | Session A - GRAD P18 (Board 18) | p. 83  
[Lydia Cates](#) | Session A - GRAD P19 (Board 19) | p. 84  
[Jeremy Cattoor](#) | Session A - UG P6 (Board 6) | p. 47  
[Maxine Cañelas](#) | Session A - GRAD P28 (Board 28) | p. 113  
[Isabella Cevallos](#) | Session B - GRAD P16 (Board 16) | p. 85  
[Nishtha Chandal](#) | Session A - PD P41 (Board 41) | p. 153  
[Sneha Chandrashekar](#) | Session C - UG P12 (Board 12) | p. 66  
[Nazmul Rahman Chowdhury](#) | Session B - GRAD P12 (Board 12) | p. 72  
[Priya Christensen](#) | Session B - GRAD P33 (Board 33) | p. 131

[Noemie Clemenceau](#) | Session A - UG P8 (Board 8) | p. 54  
[Edidiong Cornel](#) | Session A - UG P9 (Board 9) | p. 55  
[Faith Cox](#) | Session B - GRAD P13 (Board 13) | p. 73  
[Darla Delozier](#) | Session C - GRAD P28 (Board 28) | p. 132  
[Pawan Devkota](#) | Session A - PD P42 (Board 42) | p. 154  
[Pragati Dhingra](#) | Session A - GRAD P30 (Board 30) | p. 115  
[Soumita Dutta](#) | Session A Res 47 (Board 47) | p. 168  
[Chigemezu Eke](#) | Session A - UG P10 (Board 10) | p. 56  
[Oscar Villasana Espinosa](#) | Session C - GRAD P36 (Board 36) | p. 150  
[Bryan Estrada](#) | Oral presentation | p. 23  
[Anna Evers](#) | Session A - GRAD P20 (Board 20) | p. 91  
[Tristan Flores](#) | Session B - GRAD P26 (Board 26) | p. 116  
[Kaitlyn Foerster](#) | Session A - UG P4 (Board 4) | p. 38  
[Michael Fowler](#) | Session A - UG P11 (Board 11) | p. 57  
[Lauren E. Fuess](#) | Oral presentation | p. 14  
[Kimberly Garcia](#) | Session C - GRAD P29 (Board 29) | p. 133  
[Sadie Gaskins](#) | Session C - GRAD P30 (Board 30) | p. 134  
[Kavin Gidvani](#) | Session B - UG P8 (Board 8) | p. 58  
[Pratyusha Gorige](#) | Session C - GRAD P32 (Board 32) | p. 135  
[Brice Gorman](#) | Session A - GRAD P35 (Board 35) | p. 137  
[Caylah Griffin](#) | Session B - GRAD P27 (Board 27) | p. 117  
[Alannah Grimes](#) | Session B - UG P1 (Board 1) | p. 39  
[Christian Guerrero](#) | Oral presentation | p. 10  
[Jeremy Hernandez](#) | Session B - UG P2 (Board 2) | p. 40  
[Rian Hernandez](#) | Session A - GRAD P36 (Board 36) | p. 138  
[Fatima Hisam](#) | Session B - GRAD P28 (Board 28) | p. 118  
[Amber Holley](#) | Session B - GRAD P17 (Board 17) | p. 86  
[Isabella Howard](#) | Session A - UG P7 (Board 7) | p. 48  
[Kyleigh Howard](#) | Session B - GRAD P14 (Board 14) | p. 74  
[Elizabeth Jeffery](#) | Session B - GRAD P24 (Board 24) | p. 107  
[Donghoon Alex Kang](#) | Session A - PD P43 (Board 43) | p. 155  
[Anand B. Karki](#) | Session B Res P43 (Board 43) | p. 169  
[Taryn Keyzer](#) | Session A - GRAD P37 (Board 37) | p. 139  
[Pratick Khara](#) | Session B Res P44 (Board 44) | p. 170  
[Ashley Kidwell](#) | Session A - GRAD P38 (Board 38) | p. 140  
[Myungji Kim](#) | Session B - PD P39 (Board 39) | p. 156  
[Benjamin Klemp](#) | Session A - GRAD P39 (Board 39) | p. 141  
[Anisha Reddy Konakalla](#) | Session C - GRAD P26 (Board 26) | p. 121  
[Sribhavya Koneti](#) | Session B - UG P9 (Board 9) | p. 59  
[Cora Kosnik](#) | Session B - GRAD P25 (Board 25) | p. 108  
[Anne-Marie Krachler](#) | Oral presentation | p. 20  
[Santosh Kumar](#) | Session B Res P45 (Board 45) | p. 171  
[Caroline Laughlin-Black](#) | Session B – PD P40 (Board 40) | p. 157  
[Aryah Lee](#) | Session B - GRAD P15 (Board 15) | p. 75  
[Hye Seung \(Lucy\) Lee](#) | Session B - GRAD P18 (Board 18) | p. 87  
[Jun Sheng Lee](#) | Session C - GRAD P17 (Board 17) | p. 88

[Samuel Li](#) | Session B - GRAD P34 (Board 34) | p. 142  
[Alice Liu](#) | Session B - UG P4 (Board 4) | p. 49  
[Mariana Lozano-Cavazos](#) | Session B - GRAD P35 (Board 35) | p. 143  
[Alex Luecke](#) | Session C - GRAD P23 (Board 23) | p. 109  
[Tuhina Maity](#) | Session A - GRAD P21 (Board 21) | p. 92  
[Abu Amar Al Mamun](#) | Session A Res P43 (Board 43) | p. 164  
[Angelina Marranzini](#) | Session B - UG P3 (Board 3) | p. 41  
[Hannah Martinez](#) | Session C - GRAD P18 (Board 18) | p. 89  
[Lyudmyla V. Marushchak](#) | Session B - PD P41 (Board 41) | p. 158  
[Prachi Mathur](#) | Session C - GRAD P13 (Board 13) | p. 76  
[Rina Matsuda](#) | Oral presentation | p. 30  
[Ashley McDonald](#) | Session C - UG P1 (Board 1) | p. 42  
[Mithun Mekala](#) | Session C - UG P2 (Board 2) | p. 43  
[Laurissa Miller](#) | Session C - GRAD P14 (Board 14) | p. 77  
[William Miller](#) | Oral presentation | p. 32  
[Marta Cano Minarro](#) | Session A - GRAD P15 (Board 15) | p. 71  
[Jeremiah Minich](#) | Session B Res P46 (Board 46) | p. 172  
[Brittney Moser](#) | Session C - GRAD P24 (Board 24) | p. 110  
[Sruthi Mudunuri](#) | Session A - GRAD P22 (Board 22) | p. 93  
[Blake Neil](#) | Session B - GRAD P36 (Board 36) | p. 144  
[Tessa Nester](#) | Session C - GRAD P15 (Board 15) | p. 78  
[Thu Nguyen](#) | Session B - GRAD P29 (Board 29) | p. 119  
[Bryce Nielson](#) | Session C - GRAD P25 (Board 25) | p. 120  
[Vicky Nieniel](#) | Session B - UG P10 (Board 10) | p. 60  
[Stephnie Nwaiwu](#) | Oral presentation | p. 7  
[Abiola Obawemimo](#) | Session A - GRAD P23 (Board 23) | p. 94  
[BARUTI OREDY](#) | Session B - GRAD P19 (Board 19) | p. 95  
[Samuel Osei](#) | Oral presentation | p. 12  
[Oliyad Jeilu Oumer](#) | Session B - PD P42 (Board 42) | p. 159  
[Damilola Oyeboode](#) | Oral presentation | p. 22  
[Linzee Pacheco](#) | Session B - UG P5 (Board 5) | p. 50  
[Trusha Parekh](#) | Session C - PD P37 (Board 37) | p. 160  
[Nikhil Patel](#) | Session B - UG P11 (Board 11) | p. 61  
[Ananya Pathak](#) | Session C - UG P8 (Board 8) | p. 62  
[Andrew Petter](#) | Session C - PD P38 (Board 38) | p. 161  
[Christine R. Ponce](#) | Session B - GRAD P37 (Board 37) | p. 145  
[Carter Pruitt](#) | Session C - UG P9 (Board 9) | p. 63  
[Md Siddiqur Rahman](#) | Session B - GRAD P20 (Board 20) | p. 96  
[Julian Ramirez](#) | Session C - UG P3 (Board 3) | p. 44  
[Sadia Islam Reya](#) | Session B - GRAD P38 (Board 38) | p. 146  
[Samantha Ritter](#) | Session C - GRAD P16 (Board 16) | p. 79  
[Jace Salgado](#) | Session B - GRAD P21 (Board 21) | p. 97  
[Adolph Salinas](#) | Session B - GRAD P22 (Board 22) | p. 98  
[Conner Schultz](#) | Session C - UG P10 (Board 10) | p. 64  
[Salma Waheed Sheikh](#) | Oral presentation | p. 16  
[Sabona Simbassa](#) | Oral presentation | p. 24

[Mackenzie Simms](#) | Session B Res P47 (Board 47) | p. 173  
[Brent W. Simpson](#) | Oral presentation | p. 8  
[Ryan Sloan](#) | Session C - GRAD P33 (Board 33) | p. 147  
[Brianna Smith](#) | Session C - UG P11 (Board 11) | p. 65  
[Ella Stanley](#) | Oral presentation | p. 5  
[Trinity Storr](#) | Session B - UG P6 (Board 6) | p. 51  
[Teresa Sullivan](#) | Session C - GRAD P20 (Board 20) | p. 99  
[Jyoti Tamang](#) | Session C Res P41 (Board 41) | p. 174  
[Vikram Thakur](#) | Session C - PD P39 (Board 39) | p. 162  
[Nathaniel Thomas](#) | Session C - GRAD P34 (Board 34) | p. 148  
[Maria Fernanda Vaca-Riofrio](#) | Oral presentation | p. 18  
[Satya Vedula](#) | Session C - GRAD P35 (Board 35) | p. 149  
[Luis Alberto Vega](#) | Session C Res P42 (Board 42) | p. 175  
[Geetanjali Venna](#) | Session C Res P43 (Board 43) | p. 176  
[Ashley Vu](#) | Session C Res P44 (Board 44) | p. 177  
[Jordan Walker](#) | Oral presentation | p. 29  
[Meggie Wang](#) | Session C - GRAD P21 (Board 21) | p. 100  
[Jacob J. Williams](#) | Oral presentation | p. 25  
[Emma Winn](#) | Session C - GRAD P27 (Board 27) | p. 122  
[Qi Xu](#) | Session C - PD P40 (Board 40) | p. 163  
[Zhaohan Xu](#) | Session C Res P45 (Board 45) | p. 178  
[Pallavi Yadav](#) | Session C Res P46 (Board 46) | p. 179  
[Javier Zavala](#) | Session C - UG P4 (Board 4) | p. 52  
[Jiselle Zavala](#) | Session C - GRAD P22 (Board 22) | p. 101  
[Jing Zhang](#) | Oral presentation | p. 28  
[Wei Zhao](#) | Oral presentation | p. 4  
[Qing Zhou](#) | Session C Res P47 (Board 47) | p. 180  
[Duolong Zhu](#) | Session A Res 46 (Board 46) | p. 167