Texas Branch
AMERICAN SOCIETY FOR MICROBIOLOGY

2024 FALL MEETING

Galveston, TX
Nov 7 to Nov 9, 2024

Hosted by
The University of Texas Medical Branch
301 University Blvd,
Galveston, TX, 77555

ASM Branches

utmb Health

utmb Health
Microbiology & Immunology

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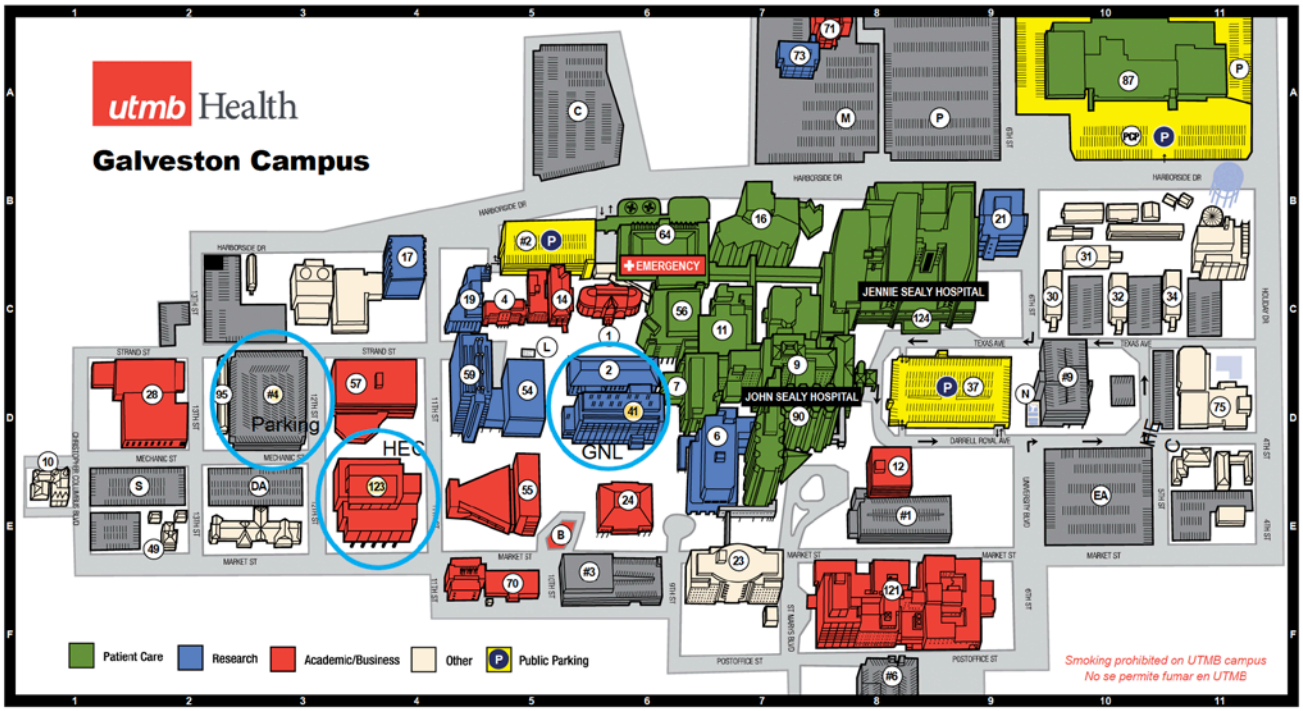
Past Texas Branch ASM Presidents

- James Stewart 1999-2001
- Karl Klose (University of Texas Health Science Center at San Antonio) 2001-2003
- Robert McLean (Texas State University at San Marcos) 2003-2005
- Heidi Kaplan (University of Texas Medical School at Houston) 2005-2007
- Poonam Gulati (University of Houston Downtown) 2007-2009
- Marvin Whiteley (University of Texas at Austin) 2009-2011
- Todd Primm (Sam Houston State University) 2011-2013
- Kendra Rumbaugh (Texas Tech University Health Sciences Center) 2013-2015
- Ali Azghani (University of Texas at Tyler) 2015-21017
- Lee Hughes (University of North Texas) 2017-2019
- Madhusudan Choudhary (Sam Houston State University) 2019-2021
- Kelly Palmer (University of Texas at Dallas) 2022-2023

THE VENUE



Building	Code	Location
1003 Market	70	E5
Administration Building	12	D8
Alumni Field House	75	D11
Ashbel Smith Building	1	C6
Basic Science Building	54	D5
Bethel Hall	32	C10
Brackenridge Hall	30	C10
Clay Hall	31	C10
Clinical Sciences Building	**7**	**D7**
Emergency Room	**64**	**B6**
Facilities Support Building	95	D3
Gleaves T. James Rose Garden	B	E5
Galveston National Laboratory	41	D6
Health Education Center	123	E3
Jennie Sealy Hospital	**124**	**C8**
John Sealy Annex	**9**	**D7**
John Sealy Hospital	**90**	**D7**
John W. McCullough Building	**11**	**C7**
Keiller Building	2	C6
Lee Hage Jamail Student Center	14	C5
Levin Hall	55	E5
Marvin Graves Building	4	C5
Mary Moody Northen Pavilion	19	C5
Materials Management Warehouse	28	D2
Maurice Ewing Hall	71	A8
Medical Research Building	59	D5
Moody Medical Library	24	E6
NMR Dockside Building	73	A7
Parking—Employee	E	E10
Parking—Employee	S	E2
Parking—Employee	DA	E3
Parking—Employee	C	A6
Parking—Employee	P	A8/11
Parking—Employee	M	A7
Parking—Employee	#3	E6
Parking—Employee	#9	D10
Parking—Employee	#6	F8
Parking—Employee	#1	E8
Parking—Employee/Student	#4	D3
Parking—Public, Hospital Garage	**37**	**D9**
Parking—Public, Clinics/ER	**#2**	**B5**
Parking—Public, PCP Lot	**PCP**	**A10**
Pat and Fred Burns Fountain	N	D9
Primary Care Pavilion (PCP)	**87**	**A10**
R. Waverley Smith Pavilion	47	D8
Research Building 6	6	E7
Rebecca Sealy	121	F8
Research Building 17	17	C4
Research Building 21	21	B9
Ronald McDonald House	10	E1
Rosenberg House	49	E2
School of Health Professions	57	D4
School of Nursing	57	D4
Shriners Hospital for Children	23	E7
TDCJ Hospital	**16**	**B7**
UTMB Health Clinics	**56**	**C6**
Vinsant Hall	34	C11
Wortham Fountain	L	C5

To see maps for all UTMB campuses and locations, visit www.utmb.edu/map

The TX ASM conference will take place on the UTMB Galveston Campus, in the Health Education Center (HEC). All sessions will be held in this building.

Free parking is available on campus for the entire duration of the conference. It is openly available and does not require prior approval nor documentation for access.

Optional GNL tours are available to registered participants. The GNL is also located on campus, a couple of buildings away from the HEC.

UTMB

UTMB opened in 1891 as the nation's first public medical school and hospital under unified leadership—already a pioneer. What began as one hospital and medical school building in Galveston is now a major academic health sciences center of global influence; a world-renowned research enterprise; and a growing, comprehensive health system with hospitals on four campuses and a network of clinics.

UTMB has a $4.9 billion annual statewide economic impact, in terms of business volume, personal income and durable goods purchases. More than 46,000 jobs in Texas are directly or indirectly attributed to UTMB.

UTMB includes schools of Medicine, Nursing, Health Professions, Public and Population Health, and Graduate Biomedical Sciences. It has four institutes for advanced study; a major medical library; a network of hospitals and clinics that provide a full range of primary and specialized medical care; and numerous research facilities. UTMB is a part of The University of Texas System and a member of the Texas Medical Center.

GNL

The Galveston National Laboratory (GNL) is a sophisticated high containment research facility that serves as a critically important resource in the global fight against infectious diseases. The GNL is located on the campus of the University of Texas Medical Branch and operates under the umbrella of UTMB's Institute for Human Infections and Immunity.

The National Institute of Allergy and Infectious Diseases (NIAID) provides partial funding for the BSL4 laboratories and operations at the GNL, and the lab's top priority is research to develop diagnostics, therapeutics and vaccines to combat emerging and re-emerging diseases that threaten public health, not only in our country, but around the world.

The Microbiology & Immunology Department

Currently chaired by Dr. Scott Weaver, UTMB's Department of Microbiology and Immunology is actively researching preventions and cures for both common and emerging infectious diseases.

Pathogens causing emerging and tropical diseases remain major threats to human health, as underscored by recent outbreaks of Ebola virus, MERS coronavirus as well as Zika and chikungunya virus. Most recently, our department has played a leading role internationally in the emergency responses to the COVID-19 pandemic. Other major infectious disease threats include resistance of bacteria to antibiotics and impacts on the human microbiota, which can affect many diseases. The continuing burdens of parasitic diseases such as Chagas, leishmaniasis also underscore the global nature of infectious disease challenges. The Department of Microbiology and Immunology (M&I) at UTMB focuses on these and many other acute and chronic infections, as well as on basic immunology. We also make major contributions to the education of graduate, medical and postdoctoral trainees in diverse fields of microbiology and immunology to develop the next generation of scientists and physicians to address these formidable public health challenges.

M&I has been a leading, research-intensive basic science department at UTMB for several decades. Our world-class faculty includes over 30 primary appointments actively engaged in research and teaching, as well as a similar number of jointly appointed members from various basic science and clinical departments across the campus. Major foci of research include the molecular and cellular basis of host-pathogen interactions, vaccine and therapeutic development, and immune mechanisms of protection. These approaches are used for a wide range of parasitic, bacterial, and viral infections, with emphasis on vector-borne and other emerging, zoonotic diseases.

Our unique high-containment capabilities and facilities, including the Galveston National Laboratory with major BSL3 and BSL4 capabilities and state-of-art core and regulatory capabilities, facilitate our major contributions to translational biodefense and emerging infectious disease research on many of the National Institute for Allergy and Infectious Disease (NIAID) priority pathogens. Finally, we are extensively integrated with the UTMB Institutes for Human Infections and Immunity, Translational Sciences, and Drug Discovery, as well as the Centers for Biodefense and Emerging Infectious Diseases and Tropical Diseases, and the Sealy Institute for Vaccine Sciences and Structural Biology/Molecular Biophysics to catalyze interdisciplinary infectious disease research across the UTMB campus.

Fall 2024 Texas Branch ASM Meeting

Day 1, Nov 7

Locations: Health Education Center & Galveston National Laboratory, UTMB Galveston Campus

10:00am-4pm	Registration (HEC 1st Floor)
01:30-02:30pm	Galveston National Laboratory Tour 1 (Optional – registered participants only)
01:30-02:30pm	Galveston National Laboratory Tour 2 (Optional – registered participants only)
05:00-05-20pm	Opening: **Sunhee Lee**, UTMB, Organizer; **Maureen Laroche**, UTMB, Organizer, **Gregory Frederick**, President, Texas Branch ASM
05:20-05:30pm	Speaker Introduction: Maureen Laroche, Organizer, UTMB (HEC 1.200/1.202)
05:30-06:30pm	Opening Keynote: **Job Lopez**, Baylor College of Medicine *Delineating the maintenance mechanisms of tick-borne pathogens through functional Genomics*
07:00-09:00pm	Networking event – appetizers and drinks served (HEC 1.200/1.202)
08:00-09:00pm	Business Meeting – Open to all members (HEC 3.201) **Join the meeting now** Meeting ID: 242 019 970 657 Passcode: GNbjg6

Day 2, Nov 8

Location: Health Education Center, UTMB Galveston Campus

07:30-11:00am	Registration (HEC 1st Floor)
7:00-8:00	Breakfast (HEC 1.200/1.202)
08:00-08:30am	2024 Eugene and Millicent Goldschmidt Faculty Mentoring Award Recipient **Indira Mysorekar**, Baylor College of Medicine *My Journeys with three microbes in the reproductive and urinary tract*
08:30-09:00am	2024 Eugene and Millicent Goldschmidt Graduate Student Award Recipient **Taylor Ranson**, Texas State University *Spaceflight-induced changes in mixed culture biofilm structure*
09:00-09:15am:	Break
09:15-11:15am:	Graduate and Undergraduate Parallel Sessions

Graduate Students – Medical Microbiology (HEC 3.206)

09:15-09:30	**Veerakit Vanitshavit**, TAMU, Sulforaphane suppresses the genotoxin colibactin production in *E. coli:*
09:30-09:45	**Qi Xu**, RICE: Role of R5 pyocin in ST111 dominance and context-dependent sensitivity of waaL deletion mutants.
09:45-10:00	**Caroline Black**, TTU: Environments Mimicking Host Infection Sites Influence Antimicrobial Susceptibility Results
10:00-10:15	**Andrej Momiroski**, TAMU: Development of a novel in silico LPS locus typing system for *Burkholderia cenocepacia* and *Burkholderia multivorans* with promising potential for the systematic prediction of phage sensitivity.
10:15-10:30	**Sofia Tamayo**, UT Tyler: Modulation of Pulmonary Epithelial Paracellular Permeability by Pneumolysin from *Streptococcus pneumoniae*.
10:30-10:45	**Blake Neil**, UTMB: Transcriptomic analysis of *Aeromonas dhakensis* in vitro and in vivo cultures: the discovery of novel virulence genes.

10:45-11:00 **Tuhina Maity**, UT Dallas: Bicarbonate Impairs the Evolution of Antibiotic Resistance in *Pseudomonas aeruginosa.*

11:00-11:15

Graduate Students – General Microbiology (HEC 3.222)

09:15-09:30 **Akanksha Varshney**, UT Austin: Trans-translation inhibitors kill *Mycobacterium tuberculosis* and pathogenic non-tuberculous mycobacteria

09:30-09:45 **Ayesha Mahmood**, UT Austin: Protein folding elements enable genetic innovation in antibiotic resistance genes.

09:45-10:00 **Braden Hanson**, TAMU: Inhibition of Uropathogens by Boosting Toxicity of Endogenous Copper

10:00-10:15 **Robert Garcia**, TAMU SA: Understanding the Abiotic Impacts of Land Development on Soil Micro diversity.

10:15-10:30 **Khondakar Sayef Ahammed**, UTHealth Houston: Fungi of the order Mucorales express a “sealing-only” tRNA ligase.

10:30-10:45 **Alex Luecke**, TTU: Transcriptomic analysis of a temperature-dependent regulatory protein in Pseudomonas aeruginosa biofilms

10:45-11:00 **Nora Bleth**, TAMU Corpus Christi: The whodunit of poop: Microbial source tracking uncovers the drivers of fecal pollution in Baffin Bay, Texas.

11:00-11:15 **Salma Waheed Sheikh**, TTU: Role of GPI8 in Regulating Surface Molecules, Toxin Resistance, and Virulence in L. major: Insights from GPI Anchor Deficient Mutants.

Undergraduate Students (HEC 3.201)

09:15-09:30 **Nancy Tran**, UT Dallas: Optimization for PhotoThermal Therapy for Enhanced Immunogenic Cell Death.

09:30-09:45 **Yanhan Deng**, RICE: *Pseudomonas aeruginosa* Sensitivity to R Pyocins Depends on Disease Presentation.

09:45-10:00 **Afzila Bhojani**, UH: CXCR3 Antagonism Reduces Zika and West Nile Virus Replication Across Various Cell Types.

10:00-10:15 **Magdalen Marston**, Sam Houston State University: DENN Domain-Containing Protein 5A/B, A Binding Partner of Daam1 Plays Critical Role During Non-Canonical Wnt Signaling and Zebrafish Development

11:15-11:30am Break

11:30am-01:30pm Lunch, poster sessions, vendor tables (HEC 1st Floor Hallway, HEC 1.200/1.202)

11:30-12:30 Poster Session A

12:30-01:30 Poster Session B

01:30-02:00pm Break

02:00-03:00pm Graduate and Undergraduate Parallel Sessions

Graduate Students – Medical Microbiology (HEC 3.206)

02:00-02:15 **Sabona Simbassa**, UTHealth Houston: Exploring the mechanism of host immune-modulated esophageal gland-dependent schistosome development.

02:15-02:30 **Amber Holley**, UTMB: MALDI-TOF mass spectrometry as a surveillance tool of mosquito-borne viruses

02:30-02:45 **Angel Elma Abu**, UTMB: Low humidity enhances Zika virus infection and dissemination in Aedes aegypti mosquitoes

02:45-03:00

Graduate Students – General Microbiology (HEC 3.222)

02:00-02:15 **Syed Raza**, UT Dallas: Exploring and exploiting synergy between macrolides and tetracyclines against *A. baumannii*

02:15-02:30 **Tetyana King**, UHCL: Comparison of MALDI-TOF MS and Whole Genome Sequencing for Identification Vibrio parahaemolyticus Strains Isolated from Oysters

02:30-02:45 **Regina Solomon**, UTMB: *Ehrlichia chaffeensis* TRP120-mediated NFAT signaling and chemokine expression

02:45-03:00

03:00-03:15pm Break

03:15-5:30pm Thematic Parallel Sessions (* indicates faculty presenters)

Vector-borne diseases & Parasitology – Chairs: Maureen Laroche & Nisha Garg (HEC 3.201)

03:15-03:45 **Shannan Rossi***, UTMB: Studying Testicular Viral Infections: From Soup to Nuts.

03:45-04:00 **Alexander Kneubehl:** BCM, Comparative Genomics of Soft Tick-borne Relapsing Fever Spirochete Genomes Yields New Insights into Plasmid Diversity and Antigenic Variation Systems

04:00-04:15 **Cusi Ferradas**, UTMB: Multidimensional approach for the assessment of the risk of Ehrlichia canis infection in Iquitos, Peru

04:15-04:30 **Margaret Becker**, UTMB: Reduced microbe abundance in an urban larval development container increases Aedes aegypti susceptibility to Zika virus

04:30-04:45 **Allison Wyrick**, UTMB: Role of Morrbid-3 lncRNA in signaling macrophage proinflammatory response for control of *Trypanosoma cruzi* infection.

Antimicrobial Resistance & Microbiome Research (HEC 3.206)

03:15-03:45 **Matthieu Gagnon***, UTMB: Structural bases of bacterial persistence and antibiotic resistance.

03:45-04:00 **Nikol Kaderabkova**, UT Austin: The natural reservoirs of Mobile Colistin Resistance proteins

04:00-04:15 **Souvik Bhattacharyya***, UTHealth Houston, Iron Memory in *E. coli*

04:15-04:30 **Alex Kang**, Houston Methodist Research Institute: Genetic Basis of Cefiderocol Nonsuceptibility in Pseudomonas aeruginosa

04:30-04:45 **Sanjay Singh**, UT Tyler: Imipenem-relebactam to restore efficacy of first-line drugs and develop novel combination regimens to treat multidrug-resistant tuberculosis

04:45-05:00 **Nicholas Dillon***, UT Dallas: More Than Just a Buffer: Bicarbonate Dictates Antibiotic Activities, Susceptibilities, and Resistance

Environmental Microbiology (HEC 3.222)

03:15-03:45 **Sarah Hu***, TAMU: Microbial eukaryotic contributions to the food web at deep-sea hydrothermal vents.

03:45-04:15 **Candice Lumibao***, TAMU Corpus Christi: Soil microbial community responses to long-term environmental disturbances.

04:15-04:30 **Claudia Trujillo**, UTMB: High Prevalence of Avian Influenza Viruses in Vietnam's Live Bird Markets.

04:30-04:45 **Smita Pal**, TAMU Galveston: Cyanotoxin negatively associates with microbial functional redundancy in lakes of the southcentral USA.

04:45-05:00 **Tanya Brown***, UT Tyler, Do Cnidarians remember previous foe attacks?

05:00-05:15 **Mohammad Kamruzzaman**, UT Arlington: Roles of Two Extracytoplasmic Function Sigma Factors and a Signal Peptide in Symbiotic Nitrogen Fixation and Desiccation Stress Response of *Bradyrhizobium japonicum*.

05:15-05:30 **Jessica Labonte*,** TAMU Galveston: Interactions between cyanotoxin producers and degraders in twenty lakes across southcentral USA

06:00pm-08:30pm Dinner and Awards (HEC 1.200/1.202)

06:00 Sit down Dinner

07:00-08:00 ASM Distinguished Speaker: **Vanessa Sperandio**, University of Wisconsin-Madison
The highs and lows of pathogen-microbiota-host interactions

08:30 Presentation and Poster Awards for Students

Day 3, Nov 9

7:30-8:30am Breakfast (HEC 1.200/1.202)

08:30-11:30am Thematic Parallel Sessions

Fungal Infections (HEC 3.206)

08:30-09:00 **Christian Perez***, UTHealth Houston: Regulatory systems controlling *Candida albicans*-host interactions
09:00-09:15 **Alison Coady***, UTMB: Protection vs Pathogenesis: Understanding the role of the antimicrobial peptide cathelicidin during fungal sepsis
09:15-09:30 **Diana Proctor***, UTHealth Houston: Human skin as a reservoir for *Candida auris* and ESKAPE pathogens: transmissible microbes and antibiotic resistance in nursing homes
09:30-09:45 **Lizette Rios**, UTMB: Cathelicidin-Mediated Modulation of Cardiac Immune Response during Fungal Sepsis

Viral and Bacterial Pathogenesis – Chair: Lisa Hensley (HEC 3.222)

08:30-09:00 **Marvin Whiteley***, Georgia Tech: Characterizing bacterial behavior during human infection to guide new discoveries
09:00-09:15 **Robert Orchard***, UT Southwestern: Identification of Trim47 as a strain specific restriction factor for murine norovirus infection
09:15-09:30 **Ngan Fung Li**, BCM: Detection of macrophage phagocytosis in human norovirus infection using ex vivo human intestinal enteroids-immune cell coculture system
09:30-09:45 **Trevor Romsdahl**, UTMB: Ebola infection activates lipid pathways for neutral lipid synthesis and lipid mediators in CD8+ cells of *Mucaca mulatta*
09:45-10:00 **Andrew Pountain***, UTHealth Houston: Transcriptional heterogeneity in microbial gene regulation and pathogenesis

10:00-10:15am Break

10:15-10:45am A word from the President: **Theresa M. Koehler**, McGovern Medical School, UT Houston
Driving Microbial Sciences Forward: ASM's Strategic Roadmap and Career Development Opportunities

Join Zoom Meeting
https://zoom.us/j/96278245597?pwd=KQKnj5f4IOEUS2MQFbkT8wySPW6x0m.1

Meeting ID: 962 7824 5597
Passcode: 188668

10:45-11:30am Panel Discussion: *Leveraging experiences from industry and government position to reach academic research goals*. Panelists: Theresa Koehler (UT Houston), Lisa Hensley (USDA), Jose Pietri (Purdue University), Matt Mendoza (UTMB).

11:30am-12:00pm Closure of the Meeting (HEC 1.200/1.202)

11:30-11:45 Postdoctoral Awards
11:45-12:00 Acknowledgements

OPENING KEYNOTE – Dr. Job Lopez, Ph.D

Dr. Lopez is currently an Associate Professor in the Section of Tropical Medicine in the Department of Pediatrics at Baylor College of Medicine. He obtained his PhD at Washington State University in Microbiology and Immunology focusing on the identification of vaccine candidates. His postdoctoral training was conducted at the Laboratory of Zoonotic Pathogens, Rocky Mountain Labs, National Institutes of Health. He started his lab at Mississippi State University and was subsequently recruited to Baylor College of Medicine (BCM). At BCM he heads the Vector Biology and Bacterial Pathogens Lab in the National School of Tropical Medicine. Job conducts research in infectious diseases, the development of molecular diagnostics, entomology, and infectious disease ecology.

The Laboratory for Vector Biology and Bacterial Pathogens has two primary focus areas:

1. **Utilizing Molecular Tools, Functional Genomics, and Animal Models to Investigate Vector-Pathogen Interactions**
   This project builds on recent work reporting tick transmission of relapsing fever spirochetes and genome sequencing of both the pathogen and tick vector. Next generation and third generation sequencing technologies were used to investigate a number of outstanding questions in the field. Transcriptional studies were also performed on the tick to further understand how relapsing fever spirochetes adapt to the arthropod vector. The goal by utilizing whole genome and transcriptomic analyses is to better understand vector-pathogens interactions and identify novel areas of intervention for both the vector and pathogen.

2. **Defining the Ecology of Pathogens Transmitted by Argasid Ticks**
   Argasid (soft bodied) ticks not only transmit relapsing fever spirochetes but also African swine fever virus, an emerging and highly contagious pathogen with high mortality rates in domestic pigs. Through multi-institutional collaborations, Dr. Lopez' lab utilizes diagnostic assays to evaluate mammalian exposure to soft ticks and the pathogens they transmit. Moreover, they are investigating the distribution of argasids through population genetic studies and their maintenance in nature. These projects will define the disease burden and ecology in regions of the globe where the pathogens are ignored.

ASM DISTINGUISHED SPEAKER – Dr. Vanessa Sperandio, Ph.D

Vanessa Sperandio, Ph.D., is Chair of the Department of Medical Microbiology and Immunology and Robert Turell Professor in infectious diseases at the University of Wisconsin-Madison. Previously, she was the Jane and Bud Smith Distinguished Chair in Medicine and professor in UT Southwestern Departments of Microbiology and Biochemistry. She received her bachelor's, master's and Ph.D. at UNICAMP in Brazil and did her post-doctoral training at the University of Maryland School of Medicine.

Sperandio was a Latin-American Pew fellow in biomedical sciences, an Ellison Foundation New Scholar and a Burroughs Wellcome Fund Investigator in the Pathogenesis of Infectious Diseases. She has been a National Academy Kavli Frontiers of Science fellow since 2007. She served on the national advisory committee of the Pew Latin-American Fellows Program and Projeto Serrapilheira in Brazil. She served on the advisory committee for the Burroughs Wellcome Fund's Investigators in the Pathogenesis-of-Infectious Diseases.

She has served as Chair of ASM Press, Chair of ASM's Division D, member of the ASM Education Awards Committee and member and Chair of the ASM Microbe Program Committee HMB-track. She currently serves on the editorial boards of mBio and Infection and Immunity and recently served on the Journal of Bacteriology editorial board (term ended in 2024).

In 2013, Sperandio was elected a fellow of the American Academy of Microbiology (AAM) and is currently serving as Chair of the AAM Academy Governors. She received the 2015 ASM Eli-Lilly and Company-Elanco Research Award and was a winner of the GSK- Discovery-Fast-track challenge in 2014. She was elected as an American Association for the Advancement of Sciences (AAAS) fellow in 2022.

Sperandio's research investigates chemical, stress and nutritional signaling at the interface among the mammalian host, beneficial microbiota and invading bacterial pathogens. The main tenet of her research is the study of how bacterial cells sense several mammalian neurotransmitters leading to rewiring and reprogramming of bacterial transcription toward host and niche adaptation. She has also identified several bacterial receptors to mammalian neurotransmitters and reported that pathogens hijack these interkingdom signaling systems to promote virulence expression. She also translated these basic science concepts into strategies to develop novel approaches to antimicrobial therapy.

GOLDSCHMIDT AWARD RECIPIENTS

Dr. Indira Mysorekar - 2024 Eugene and Millicent Goldschmidt Faculty Mentoring Award Recipient

I am the inaugural E.L. Wagner Endowed Professor in Infectious Diseases at Baylor College of Medicine (BCM) in the Department of Medicine, where I serve as Chief of Research in the Section of Infectious Diseases, Vice Chief of the Section, and Professor in the Huffington Center on Aging and the Department of Molecular Virology and Microbiology. My journey began in India and Tanzania before moving to Sweden for my BS and MS degrees at the University of Lund. I earned my PhD at Washington University School of Medicine in St. Louis, where I later became the James P. Crane Endowed Professor of Maternal Fetal Medicine and Director of the Center for Reproductive Health Sciences. My career has been dedicated to advancing women's and reproductive health through translational research, bridging basic and clinical science, and mentoring students and junior faculty in these fields. I have been honored with the 2024 Outstanding Woman in Science Award from AWIS, the Distinguished Service Award from the American Society for Reproductive Immunology (ASRI), I was also recently inducted as a Fellow of the American Academy of Microbiology and elected as President of ASRI. In 2021, I received the Christian J. Herr Award for outstanding achievements in reproductive immunology. Most recently, my student, Brittany Jones and I were recipients of the 2024 HHMI Gilliam Fellowship Award to mentor-mentee pairs for our proposed research and advocacy for maternal health.

My lab has made significant discoveries in urogenital tract immunology and microbiology, focusing on viral infections during pregnancy, urinary tract infections in older women, and developing therapeutic interventions for improving urogenital health. Our research has been supported by multiple NIH grants and awards from the Burroughs-Wellcome Fund and the March of Dimes. Over the past 15 years, I have mentored a diverse group of 21 undergraduates, 15 graduate students, and 18 clinical/postdoctoral fellows, many from underrepresented communities, all of whom are pursuing careers in science and women's health. I co-direct two T32 training programs at BCM and serve as senior faculty advisor for the BCM-wide K-Club, which prepares postdoctoral fellows for competitive career development awards. My commitment to mentorship and global women's health extends beyond the lab, having facilitated the development of a women's health center in Ethiopia and been named a Woman Leader in Global Health. I have been proudly recognized as Mentor of the Year in 2019 by the Washington University Graduate Student Senate, and I was also awarded an Outstanding Faculty Mentor Award there for implementing programs for sponsoring and mentoring women.

Taylor Ranson - 2024 Eugene and Millicent Goldschmidt Graduate Student Award Recipient

Taylor Ranson received her BS from Texas State University in 2022 and continued as an M.S. student with Bob McLean in the Microbiology of Biofilms and Polymicrobial Communities lab at Texas State University. Her passion for microbial physiology and biofilms began with her undergraduate research in the influence of central metabolism disruption on *Escherichia coli* biofilm formation, which resulted in her first publication. Currently, her research focuses on spaceflight-induced changes of polymicrobial biofilm structure and susceptibility to silver disinfection.

FOOD AND DRINKS

Highlighted establishments offer discounts to ASM registered participants.

On campus dining options

America's Kitchen	500 Harborside Dr
Suki Poke By The Sea	427 Market St
Gonzalo's American Bistro (10%)	415 9th St
Mom's Farm to Table (15%)	902 Postoffice St., #1
Seawall Cuisine	500 Seawall Boulevard, Ste 220
Chilango's Brothers Taco Shop	708 Holiday Dr
Dawn Donuts	6304 Stewart Rd
Smooth Tony's	902 Postoffice St
The Original Mexican Café	1401 Market St
The Sunflower Bakery	512 14th St
Mosquito Café	628 14th St

On campus drinking options

Lucky Lounge	904 Avenue M
Safari Beach Company	910 Avenue M
East End Tavern	916 Avenue M
Gonzalo's American Bistro (10%)	415 9th St
Hotel Lucine	1002 Seawall Blvd

Near campus dining options

Vida Agave	711 25th St
Miller's Seawall Grill	1824 Seawall Blvd
Gaido's Seafood Restaurant	3828 Seawall Blvd
Nick's Kitchen and Beach Bar	3802 Seawall Blvd
Mario's Seawall Italian Restaurant	628 Seawall Blvd
Pirate Island Bar & Grill (10%)	728 Seawall Blvd, Unit A
Shark Shack Beach Bar & Grill	2402 The Strand
Little Daddy’s Gumbo Bar	2107 Postoffice St
Stuttgarden Tavern	111 23rd St. Galveston
Yaga’s Café (11/8 4-7pm complimentary nachos at Tsunami Exotic Tequila Emporium)	2314 The Strand

Near campus drinking options

Hearsay On The Strand	2410 The Strand
Hendley Wine Co.	2016 The Strand
Sharky's Tavern	504 25th St,
The Proletariat Gallery & Public House	2221 Market St #100
MarMo Cafe & Lounge	2121 Market St suite 101

Sugar & Rye	2401 Church St
The Old Galveston Club	418 21st St
East End Tavern	916 Avenue M
Texas Tail Distillery	2416 Postoffice St
Tremont House	2300 Ship Mechanic Row St
Float Pool & Patio Bar	2828 Seawall Blvd
Beerfoot Beach Bar	2816 Avenue R 1/2
Buckshot Saloon	2409 Market St
Galveston Island Brewing	8423 Stewart Rd
Naked Iguana Brewery	1828 The Strand
Texas Tail Distillery	2416 Postoffice St
Devil and The Deep Brewing	2425 Postoffice St
Sky Bar (Specifically happy hour deals)	2105 Postoffice St
Brews Brothers	2404 The Strand
Stuttgarden Tavern	111 23rd St. Galveston
Daiquiri Time Out	2701 Market St
Market Station	2310 Market St

ACKNOWLEDGEMENTS

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Our profound thanks go to our administrative support team—Ana Laura Ramos, Shannon Speakes, and Tina Brasher—whose tireless dedication made this conference possible. Ana, Shannon, and Tina, your remarkable assistance, particularly in navigating this commitment on top of your already demanding roles, has been invaluable. We are deeply appreciative of how you consistently made time for us without ever making us feel like a burden. We also thank Sonia Gonzalez from the Finance Department for her assistance.

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Thank you to all for your generosity and commitment to making this event a success.

Drs. Maureen Laroche and Sunhee Lee,
Organizers of the meeting.

POSTERS

11:30-12:30 Poster Session A
12:30-01:30 Poster Session B

GENERAL MICROBIOLOGY - Undergraduate Students			
A	UGP 1	Carson Bellew	Sequencing the genomes of environmental bacteriophage samples targeting Pseudomonas Aeruginosa
B	UGP 2	Aurelio Del Carmen	Revisiting Campylobacter plasmids: a pangenome analysis
A	UGP 3	Christia DeLuna	FlyingTortilla, ScarletRaider, BluerMoon, and EvenBluerMoon: Characterization of Novel Actinobacteriophages Isolated in Lubbock, Texas
B	UGP 4	Samuel Demaio	Evaluating the Impact of Washing Techniques on Microbial Reduction and Antibiotic Resistance in Texas Blueberries
A	UGP 5	Michael Garcia	Nutrient acquisition in climate-smart soybeans enhanced by a drought-tolerant Bradyrhizobium isolate
B	UGP 6	Natalie Meklenburg	Virus-host interactions in warm monomictic lakes across southcentral USA: deciphering the potential ecological implications of viral infections
A	UGP 7	Vincent Mercado	Using Transposon Sequencing (Tn-Seq) to identify essential genes for temperature-dependent biofilm formation in Pseudomonas aeruginosa
B	UGP 8	Katelyn Perez	Investigation of Bacterial Content of a Commercial Skin Probiotic
A	UGP 9	Casey Reyes	Protocol Development for Identification of Staphylococcus aureus from Wastewater
B	UGP 10	Madison Schultz	High prevalence of Campylobacter and Enterococcus as co-contaminants in retail chicken liver products.
A	UGP 11	Aleck Servin	Bioinformatic Analysis of Rhomboid Proteases in Pathogenic Protozoa

PATHOGENIC MICROBIOLOGY - Undergraduate Students			
B	UGP 12	Layla Behrens	Developing a Model System for Campylobacter Gut Infections
A	UGP 13	Jessica Guilhas	Characterization of Novel Genes Critical for Iron Acquisition from Hemoglobin in Bacillus anthracis Sterne
B	UGP 14	Harish Jawahar	SCV Formation as a Mechanism for Nafcillin Resistance in S. Aureus
A	UGP 15	Cora Kosnick	Developing Local Delivery of Phage to Treat Antibiotic-Resistant Staphylococcus aureus Biofilm Osteomyelitis
B	UGP 16	Allison Luong	Determining DGAT2's role in maintaining lytic KSHV infection
A	UGP 17	Shannon Moncier	Modulating the Human Gut Microbiome with Gochujang, a Fermented Soybean Product
B	UGP 18	Spandan Mukherjee	Elucidating the effect of Plasticizer Phthalates (DEHP) on Kaposi's Sarcoma associated Herpesvirus Infection
A	UGP 19	Jace Salgado	Understanding Polymicrobial Competition in Chronic Wounds Using Artificial Clots in Mice
B	UGP 20	Emily Skinner	Microbial Interactions and Environmental Conditions Influence Antibiotic Susceptibility
A	UGP 21	Joshua Tadegegn	Analysis of Pneumolysin-Induced Damage and Repair in Bronchial Epithelial Cells in Vitro

B	UGP 22	Claire Wang	“Exploring the Role of Nucleotide Synthesis During KSHV Lytic Replication”
A	UGP 23	Andre Gil	Functional Study of Profilin Isoforms Using a Vertebrate Model

ENVIRONMENTAL MICROBIOLOGY – Graduate Students			
B	GSP 1	Dasire Brawley	Surveillance for reticuloendotheliosis virus and lymphoproliferative disease virus in wild turkeys
A	GSP 2	Sarthak Chaudhary	Development of a LAMP assay to detect pBI143 - an abundant plasmid specific for human waste
B	GSP 3	Clay Gabel	DFW Soil Bacteria Shed Light on Understudied Genomes
A	GSP 4	Christopher Howard	Application of Digital PCR and High-Throughput Sequencing of 16S rRNA to estimate the contribution of Human Waste to Tributaries of Galveston Bay
B	GSP 5	Yue Liu	Rhizosphere Soil Fungal Community Responses to Nitrogen Addition Reflects Both Plant Genotypic and Heritable Trait Variations
A	GSP 6	Pierce Lynch	Soil organic matter and microbial extracellular enzyme activities vary across soil infiltration berm installations
B	GSP 7	Hannah Martinez	Soil health indicators of urban green spaces: addressing microbial aspects of soil fertility
A	GSP 8	Milena Rodriguez Pilco	Viral Activity in Sediments from the South Atlantic Gyre
B	GSP 9	Austen Rowell	Using NGS RNA-Seq to determine viral communities in arthropod populations.
A	GSP 10	Eshita Shahanaz	Screening of Flies as Vectors and Sentinels of Antimicrobial Resistant Human-Foodborne Pathogens in Texas.
B	GSP 11	Angelica Torres	Effects of Bradyrhizobium Biofertilizer Application on Greenhouse Gas Emissions in South Texas
A	GSP 12	Jezreel Wilson	Diversity of soil mycobiome in the gulf coast prairie dunes of barrier islands in South Texas

GENERAL MICROBIOLOGY – Graduate Students			
B	GSP 13	Michael Awuah	Breaking Up The Break Down: How An Open Reading Frame Inhibits Lysis In Bacteriophage N4
A	GSP 14	Seerpatham Divyasorubini	Ribosomal protein bL27: A key regulator of trans-translation efficiency and antibiotic sensitivity
B	GSP 15	Ha Do	Envelope stress responses functionally coordinate to maintain cell homeostasis in Escherichia coli.
A	GSP 16	Fahareen Mosharraf	Isolation and characterization of bacteriophages infecting Pseudomonas aeruginosa: exploring ecological diversity and therapeutic potential.
B	GSP 17	Evan Ortiz	Addressing Microbial Corrosion During Spaceflight With AI-Driven Image Analysis
A	GSP 18	Anh Pham	Inhibitors of an extra-cytoplasmic function (ECF) sigma factor, σE as anti-infectives and antibiotics against Gram-negative pathogens
B	GSP 19	Irvin Rivera	Phylogenomic framework for Escherichia coli of serotype O118: Virulence gene prevalence and pathovar boundaries informed by the comprehensive profiling of 359 genomes
A	GSP 20	Musfirat Shubaita	Long chain fatty acids promote Candida albicans gut colonization

B	GSP 21	Teresa Sullivan	Elucidating resistance mechanisms to the BAM complex inhibitors.

BACTERIAL PATHOGENESIS – Graduate Students			
A	GSP 22	Bhavani Balasundarasekar	Decoding In-Host Transcriptomic Adaptations of Staphylococcus aureus in Response to Mammalian Defensins
B	GSP 23	Namrata Dinesh Bonde	Nafcillin Resistance Drives Azithromycin Sensitivity in MRSA
A	GSP 24	Stanlee Brandt	Examination of Campylobacter and Enterococcus Interactions in an Animal Infection Model
B	GSP 25	John Camp	Using GWAS to investigate how antibiotic resistance evolves in Pseudomonas aeruginosa during Cystic Fibrosis
A	GSP 26	Anna Evers	Colistin and Meropenem Seesaw Interactions in Acinetobacter baumannii
B	GSP 27	Iris George	Competitive Fitness of Asymptomatic Bacteriuria E. coli Strain 83972 Against Uropathogens in Human Urine
A	GSP 28	Raquel Luna	Staphylococcus aureus in Orthopedic Device-related Infection Biofilm Models
B	GSP 29	Valeria Melendez Hebib	Investigating the interplay between diet and the gut microbiome in the pathogenesis of necrotizing enterocolitis
A	GSP 30	Aeron Pennington	Connection between clpX and msrB and increased sensitivity to cell wall antibiotics in Bacillus anthracis Sterne
B	GSP 31	Arugonda Ravali	Impact of Bicarbonate on Pseudomonas aeruginosa virulence and host immune interactions in Cystic Fibrosis
A	GSP 32	Nathaniel Thomas	Examining how bicarbonate resistance fuels virulence in Acinetobacter baumannii
B	GSP 33	Suman Tiwari	Novel Biofilm Mutations Promote Minocycline Resistance in Acinetobacter baumannii
A	GSP 34	Tessa Williams	A Reverse Genetic Screen Elucidates the Mechanisms of Anaerobic Copper Toxicity in E.coli
B	GSP 35	Muneer Yaqub	Redefining Antibiotic Resistance in Acinetobacter baumannii

MEDICAL MICROBIOLOGY – Graduate Students			
A	GSP 36	Katherine Araya	Increased Risk of Dementia Associated with Herpes Simplex Virus Infections: Evidence from a Retrospective Cohort Study Using U.S. Electronic Health Records
B	GSP 37	Eranda Berisha	Elucidating the role of Fatty Acid Binding Proteins (FABPs) in KSHV Replication and Maximal Infectious Virion Production
A	GSP 38	William Carpenter	DEVELOPING A NOVEL MURINE MODEL OF POLYTRAUMA-ASSOCIATED MUCORMYCOSIS
B	GSP 39	Jonatham Castro	Characterizing the role of the Scp160 SESA Complex Component in Regulation of Candida albicans Virulence Properties
A	GSP 40	Garrett Cutchin	Immune correlates of memory T cells with vaccine-induced protection against fatal murine rickettsiosis
B	GSP 41	Emily Cwiklik	Cathelicidin's impact on macrophage response during infection with Candida albicans
A	GSP 42	Douglas Davis	Exploring Regulatory Mechanisms of the Essential Immediate-Early M142 gene of Murine Cytomegalovirus
B	GSP 43	Maria del Carmen	Unraveling the functional role of KSHV latent-host protein-protein interactions

A	GSP 44	Emily Hendrix	Novel live-attenuated and subunit vaccines provide long-term protection against pneumonic plague
B	GSP 45	Lyudmyla Marushchak	Molecular Typing of Adenoviruses Associated with Respiratory Illness Among Humans and Poultry, Pakistan
A	GSP 46	Raj Patel	The Search for Anti-Fungal Compounds Produced by Myxococcus xanthus
B	GSP 47	Leslie Rodriguez	Study the immune mechanisms of an insect-based Chikungunya chimera vaccine candidate -induced immune responses and safety in mice and guinea pigs
A	GSP 48	Ryan Sloan	Defining the cellular and functional heterogeneity of schistosomes' esophageal gland and associated tissues.

POST DOC			
B	PDP 1	Prasanth Manohar	Metagenomic Mining: Evolution of 'Single-Gene Lysis' Systems in Small Phages
A	PDP 2	Swara Yadav	Oxford Nanopore Technology long-read sequencing enables antimicrobial resistance predictions for emerging mechanisms of extended-spectrum beta-lactamase-producing E. coli
B	PDP 3	Michael Eledge	Virus-Induced Purinergic Signaling Amplifies Type 1 & III Interferon Production and Interferon Resposnes
A	PDP 4	Ismaila Shittu	Novel Rodent Coronavirus Detected in Beef Cattle, Mexico

FACULTY AND OTHER			
B	FP 1	Daniel Cummings	A One-Health Approach in Surveilling for Emerging Respiratory Viruses on Cattle Farms in Kentucky and Indiana
A	FP 2	Sheuli Zakia	A New Phylogenetic and Sequence Analysis of Penicillin-Binding Proteins

ORAL PRESENTATION ABSTRACTS

GRADUATE AND UNDERGRADUATE STUDENT ORAL PRESENTATION

Undergraduate Students

UGM 1

Optimization for PhotoThermal Therapy for Enhanced Immunogenic Cell Death

Nancy Tran, Ryanne Ehrman, Jeremiah Gassensmith
University of Texas at Dallas, Richardson, USA

Photothermal therapy (PTT) is a non-invasive and promising new cancer therapeutic for shallow, solid tumors—e.g., breast and melanoma. It utilizes tissue-penetrating, near-infrared (NIR) light to locally heat a tumor site in the presence of a photothermal agent (PTA). Effective PTAs convert NIR light into heat with high photothermal efficiency through strong absorption within the NIR spectrum and low fluorescence quantum yield. PTT is an attractive therapeutic for immunogenically "cold" tumors and elicits immunogenic cell death (ICD)—which is achieved through the production of damage associated molecular patterns (DAMPs). DAMPs are expressed by dying cells that provoke a pro-inflammatory immune response. ICD-invoking cancer therapies can "train" the immune system to recognize and fight cancer, aiding in treatment of both primary and metastatic tumors. To optimize the photothermal parameters of PTT, a viral PTA, PhotoPhage was synthesized through the bioconjugation of NIR-absorbing croconium dye onto bacteriophage Qß. We extensively studied PhotoPhage-mediated PTT and its ICD abilities in 4T1 triple negative breast cancer. The photothermal parameters of PhotoPhage-mediated PTT for enhanced ICD was optimized and the adjuvanting properties of PhotoPhage was studied. Mild heating, 50–60ºC was established to be optimal for achieving 4T1 cellular apoptosis and allows for the greatest expression of surface-exposed DAMPs. Additionally, the secretion of pro- and anti-inflammatory cytokines was investigated when presented with PT-treated 4T1 cells. This photothermal optimization allows us to not only study the "cold" to "hot" tumor conversion, but also ensures that we utilize PTT- mediated ICD to the maximum of its therapeutic ability.

UGM 2

***Pseudomonas aeruginosa* Sensitivity to R Pyocins Depends on Disease Presentation**

Yanhan Deng[1], Qi Xu[1,2], Liyang Zhang[1], Natasha Kirienko[1]
[1]Department of Biosciences, Rice University, Houston, USA. [2]Department of Bioengineering, Rice University, Houston, USA

Pseudomonas aeruginosa is an opportunistic pathogen commonly found in medical settings, frequently exhibiting resistance to frontline antibiotics. It causes a variety of nosocomial infections, especially in patients with preexisting health conditions such as cystic fibrosis and leukemia. Our whole-genome analysis revealed the presence of high-risk multi-locus sequence types (MLST) including ST111, ST253, and ST235. These sequence types encode an extracellular antipseudomonal complex called R pyocin and confers upon pyocin-producing strains a competitive advantage in *P. aeruginosa* intraspecific competitions by killing other strains. R pyocins can be subdivided into R1 to R5 types, with R5 having the widest killing range. In this study, we analyzed and typed 5,135 *P. aeruginosa* strains available in the Pseudomonas Genome Database (PGD), to investigate the relationship between R pyocin subtypes and the epidemiological risks associated with specific sequence types. We observed that six out of eight high-risk sequence types, including ST235 and ST111, encode R5 pyocin. To uncover the role of R pyocins in intraspecies competition, we treated *P. aeruginosa* isolates from different disease types (e.g. leukemia, cystic fibrosis, etc) with representative R1, R2, or R5 pyocins. Unexpectedly, a dramatic difference in sensitivity to R5 pyocins based on disease presentation was seen. Specifically, over 70% of cystic fibrosis isolates were sensitive to R5 pyocins, while killing ability of R5 pyocins against isolates from patients with leukemia was much lower. Our analysis of genomes of CF and leukemia *P. aeruginosa* isolates will uncover rules for sensitivity to pyocins, helping to evaluate their potential as antipseudomonal treatment.

UGM 3

CXCR3 Antagonism Reduces Zika and West Nile Virus Replication Across Various Cell Types

Afzila Bhojani[1], Marc Hanke[1], Jennifer L. Spencer Clinton[2,3]
[1]University of Houston, Houston, USA. [2]Baylor College of Medicine, Houston, USA. [3]Texas Children's Hospital, Houston, USA

Mosquito-borne flaviviruses, such as West Nile (WNV), dengue, and Zika (ZIKV) viruses cause over 400 million infections annually posing a global health concern. While most infections tend to be mild, severe cases can cause complications such as hemorrhagic fever, organ failure, and neurological damage. Currently, no antiviral agents and few vaccines are available, creating an urgency for new therapeutics to prevent flavivirus diseases. Host immune responses, including conserved anti-viral mechanisms such as interferons regulate flavivirus replication. The interferon stimulated gene, IP-10, plays a role in immune responses and signals through its receptor, CXCR3. Our previous studies showed that CXCR3 blockade reduced ZIKV replication in prostate cells. Therefore, we hypothesized that CXCR3 antagonism may have conserved effects to abrogate flavivirus replication across multiple viruses and cell types. We assessed CXCR3 expression in THP-1 monocytes, THP-1 macrophages, and HTR-8 cells using immunofluorescence and found that each cell type highly expresses the CXCR3 receptor. We then treated THP-1 monocytes with CXCR3 antagonist prior to infection with ZIKV or WNV and assessed changes in viral envelope gene expression by qRT-PCR. Antagonist-treated cells showed a significant reduction in ZIKV and WNV envelope expression compared to controls indicating that CXCR3 antiviral activity is conserved across multiple flaviviruses and cell types. Future studies will assess CXCR3 antagonism in THP-1 macrophages, HTR-8 cells, and animal models to determine if the reduction in WNV and ZIKV replication is conserved. Future research will investigate CXCR3's antiviral mechanism to aid in the development of antiviral therapeutics for flaviviruses.

UGM 4

DENN Domain-Containing Protein 5A/B, A Binding Partner of Daam1 Plays Critical Role During Non-Canonical Wnt Signaling and Zebrafish Development

Magdalen Marston, Alicia Mendoza, Isabella Simon, Sharmin Hasan
Sam Houston State University, Huntsville, USA

The Wnt family of signaling molecules plays roles in early embryonic development, including cell fate determination, proliferation, motility, and primary axis establishment. Disruption of Wnt signaling is linked to human pathologies, including birth defects, colon cancer, and neurodegenerative diseases such as Alzheimer's and Parkinson's. The Wnt pathway is categorized into canonical/β-catenin-dependent and non-canonical/β-catenin-independent pathways. The non-canonical pathway regulates neural tube closure in vertebrate embryos. Daam1 plays a role in cytoskeletal rearrangements during gastrulation. It is not fully understood how Daam1 establishes its role that makes cytoskeletal rearrangements during vertebrate gastrulation.

In a screen for Daam1 effectors, Dennd5a was identified as a Guanine Exchange Factor that activates Rab proteins. Zebrafish (*Danio rerio*) dennd5a (zdennd5a) and *Xenopus laevis* dennd5a (xdennd5a) interact and co-localize with Daam1, independent of Wnt stimulation. We found that zdennd5a and xdennd5a started temporal expression maternally from the 1-cell stage until the early embryonic stages, with morpholino knockdown studies suggesting its critical role during gastrulation.

We found that due to a genome duplication event in zebrafish, they possess a paralogue, dennd5b, which shares 68.18% sequence similarity with human DENND5B. Zebrafish *dennd5b* is expressed maternally and shows strong spatial expression during gastrulation and later stages, particularly in the brain and somites. Morpholino knockdown of *dennd5b* results in a compressed head and deformed tail, indicating its importance in vertebrate development. Current work involves mRNA over-expression and CRISPR-Cas9 knockout studies. With the over-expression, knockdown, and knockout analysis, this study better understands how *dennd5a/b* functions during early embryonic development of vertebrates.

Graduate Students – Medical Microbiology:

GSMM 1

Sulforaphane suppresses the genotoxin colibactin production in *E. coli.*

Veerakit Vanitshavit[1], Mark Aldren Feliciano[2], Brian Gold[2,3], Parinya Tipanyo[4], Sargurunathan Subashchandrabose[1]
[1]Texas A&M, College, USA. [2]New Mexico State University, Las Cruces, USA. [3]University of New Mexico, Albuquerque, USA. [4]Chulabhorn Research Institute, Bangkok, Thailand

Escherichia coli strains harboring the *pks* genomic island produce colibactin, a genotoxin, that induces DNA damage in eukaryotic cells. Increasing evidence indicates that colibactin induces colorectal tumorigenesis in humans. However, the factors influencing colibactin production in *E. coli* remain incompletely understood. We used a *pks*+ve human *E. coli* strain CFT073, and constructed a Δ*pks* mutant to use a control. Colibactin production was quantified with a synthetic probe that detected ClbP peptidase activity, which is crucial for colibactin maturation. Our findings indicated that colibactin production was highest in late stationary phase and in minimal media. By screening a collection of endogenous and dietary compounds found in the gut, we identified sulforaphane as an inhibitor of colibactin production. Sulforaphane is a dietary phytochemical derived from cruciferous vegetables. While sulforaphane is bactericidal at higher concentrations, sub-inhibitory levels of sulforaphane abrogated colibactin at transcriptional level and ClbP peptidase activity in *E. coli*. Intriguingly, sulforaphane significantly decreased DNA crosslinking *in vitro* by preventing colibactin production in *E. coli* in a dose-dependent manner. Furthermore, *in silico* modeling of ClbP with precolibactin and sulforaphane suggested that sulforaphane likely interacts with tyrosine152, part of the catalytic triad, and with the amine on precolibactin, thereby impeding precolibactin processing. In summary, sulforaphane is an effective colibactin production inhibitor in *pks*+ve *E. coli* and it decreases DNA damage caused by colibactin. Studies are in progress to translate our *in vitro* findings to animal models to evaluate the impact of sulforaphane on colibactin production and DNA damage in the gut.

GSMM 2

Role of R5 pyocin in ST111 dominance and context-dependent sensitivity of *waaL* deletion mutants

Qi Xu, Yanhan Deng, Liyang Zhang, Filemon Tan, Natasha Kirienko
Rice University, Houston, USA

Pseudomonas aeruginosa is an opportunistic human pathogen that frequently exhibits multi- or even pan-drug resistance towards most frontline antibiotics, leading to increasing infections and deaths among hospitalized patients, especially those with compromised immune systems. Intriguingly, these infections are predominantly caused by a small group of high-risk sequence types (ST), such as ST111, but the basis for this dominance remains unclear. In this study, we screened a genome-wide transposon mutant library, leading to discovery that ST111 strains outcompete multiple non-ST111 strains through production of R5 pyocin. Pyocins are phage tail-like bacteriocins produced by *P. aeruginosa* that bind to the core lipopolysaccharides (LPS) on target cells. Competitive *in vitro* dominance of ST111 was lost upon deletion of the gene for a structural component of R5 pyocin. We identified the O-antigen ligase WaaL as a key component of sensitivity to R5 pyocins. Deletion of *waaL* sensitized previously R5-resistant strains. Interestingly, sensitivity of *waaL* mutants to R1 and R2 pyocins appeared to at least partially depend on what type of pyocin the strain encoded, suggesting that interactions between pyocins and LPS are more complex than currently understood. In addition, PA14 LPS mutants with high susceptibility to R pyocins exhibited poor swimming motility, providing a phenotypic marker for pyocin susceptibility. Overall, our study sheds light on the mechanisms underlying ST111 strain dominance and highlighted the role of *waaL* in determining R5 pyocin susceptibility.

GSMM 3

Environments Mimicking Host Infection Sites Influence Antimicrobial Susceptibility Results

Caroline Black, Catherine Wakeman
Texas Tech University, Lubbock, USA

While some aspects of the host-pathogen interface are common to all infection sites, localized environments can have unique factors specific to the body site. Chronic infections are often polymicrobial in nature with great variation in microbial communities between patients. Additionally, nutrient compositions vary at infection sites across the body. Our research demonstrates that the antibiotic susceptibilities of pathogens can dramatically shift depending on nutritional composition and the presence of other microorganisms. Specifically, we demonstrate that *Enterococcus faecalis* grown in a polymicrobial community containing other common wound pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*) in Cation-Adjusted Mueller Hinton Broth (CAMHB) demonstrated increased susceptibility to gentamicin. We believe this is due to heme cross-feeding allowing more gentamicin to enter the cell via altered proton motive force. However, performing the same AST in

anaerobic conditions reversed this phenotype. When gentamicin AST was performed in media more representative of different body sites (Lubbock Chronic Wound Media (LCWM) and Synthetic Cystic Fibrosis Media 2 (SCFM2)), the AST results differed from the CAMHB results. In SCFM2, *E. faecalis* no longer demonstrated increased susceptibility in the community. However, in LCWM, *E. faecalis* displayed increased susceptibility to gentamicin regardless of community presence. When AST was performed from communities grown in mouse wounds, the AST results for *E. faecalis* showed sensitization to gentamicin. Overall, these results demonstrate that environmental conditions play a role in determining an individual bacterium's antibiotic susceptibility. By accounting for the infection environment when determining susceptibilities, we can prescribe more effective treatments and improve patient outcomes.

GSMM 4

Development of a novel *in silico* LPS locus typing system for *Burkholderia cenocepacia* and *Burkholderia multivorans* with promising potential for the systematic prediction of phage sensitivity

Andrej Momiroski, Carlos Gonzalez, Jason Gill
Texas A&M University, College Station, USA

The *Burkholderia cepacia* complex (Bcc) is a group of Gram-negative bacteria with the ability to colonize and cause severe infection in the lungs of cystic fibrosis (CF) and chronic granulomatous disease (CGD) patients. Lipopolysaccharide (LPS) comprises the majority of the outer leaflet of the outer membrane of Gram-negative bacteria, and affects sensitivity to phages. In recent years, locus typing, which involves the in silico characterization of genomic regions involved in the synthesis of LPS or other antigenic surface features, has begun replacing traditional LPS-based classification methods such as serotyping by agglutination assys. Two genomic regions in *Burkholderia cenocepacia* type strain K56-2 have been implicated in LPS synthesis. These loci are flanked by highly conserved gene "anchors," which can be used to identify and retrieve these loci from hundreds of sequenced strains in public repositories. Analysis of the gene contents at these regions in the two most common Bcc members B. cenocepacia (644 genomes) and *B. multivorans* (810 genomes) identified 36 unique LPS locus types in B. cenocepacia and 84 types in *B. multivorans*. Distribution of types was not even across strains, with 34 types accounting for ~84% of deposited strains. Twenty-three strains from the ten most common types in B. cenocepacia were tested for sensitivity to four phages and four tailocins. Two of the phages infected all tester strains of one type, and a third phage infected all tester strains of two different types, highlighting the potential for predicting a strain's phage sensitivity using that strain's LPS locus type.

GSMM 5

Modulation of Pulmonary Epithelial Paracellular Permeability by Pneumolysin from Streptococcus pneumoniae

Sofia Tamayo, Esther Nwachukwu, Dustin Patterson, Andrey Komissarov, Ali Azghani
University of Texas at Tyler, Tyler, USA

Pneumococcal diseases caused by opportunist Streptococcus pneumoniae are a major health concern worldwide with a high morbidity and mortality rate in young children and elder adults. Various virulence factors allow the organism to cause severe pulmonary infections and empyema in individuals with underlying diseases. Pneumolysin (PLY), a key virulence factor released by autolysis, is a cholesterol-dependent pore-forming cytolysin protein. We tested the hypothesis that PLY increases epithelial paracellular permeability by affecting the anatomy and physiology of epithelial tight junctions. To address this hypothesis, we cloned and expressed the PLY gene and utilized non-lytic concentrations of purified protein (rPLY), determined by LDH assay. Confluent monolayers of human airway Calu-3 cell line were cultured on porous membrane inserts and used to investigate PLY's impact on epithelial paracellular permeability. Transepithelial electrical resistance (TEER) was used to evaluate the integrity of epithelial junctional complexes. The TEER data revealed a significant reduction in resistance following treatment with various concentrations of rPLY (5, 15, and 30 µg/mL), over a period of up to 50 minutes. TEER data indicated a significant decrease in epithelial resistance at 50 minutes for the highest concentration (C:4, treated: 12, n=16, 4 independent experiment, $p<0.05$). Fluorescence microscopy images indicated subtle alterations in the intensity and localization of tight junction protein Occludin. In conclusion, our data revealed the potential mechanism of *S. pneumoniae*-induced pulmonary injury in cellular level.

GSMM 6

Transcriptomic analysis of *Aeromonas dhakensis in vitro* and *in vivo* cultures: the discovery of novel virulence genes

Blake Neil[1], Michael Netherland[2], Hasan Nur[2], Aditi FNU[1], Parimal Samir[1], Emily Hendrix[1], Gabrielle Cheney[1], Paul Kilgore[1], Jian Sha[1], Ashok Chopra[1,3]
[1]University of Texas Medical Branch, Galveston, USA. [2]EzBiome Inc., Gaithersburg, USA. [3]Institute for Human Infections and Immunity, Galveston, USA

Evidence suggests that, of the 19 *Aeromonas* species known to infect humans, *A. dhakensis* is often associated with fatal, systemic infections and high rates of antimicrobial resistance. Two strains of *A. dhakensis* were recently isolated from fatal cases of bacteremia at the University of Texas Medical Branch and we sought to obtain a global picture of the systems and pathways altered by these isolates during infection. We performed RNA sequencing on bacterial RNA isolated from cultures of each (alongside a reference strain) grown in either the peritoneal cavity of a mouse (*in vivo*), or in stagnant media (*in vitro*) for 6 hours. Transcriptomic profiles for each culture condition were annotated and compared, and relative expression values were generated using *in vitro* expression as the baseline. As validation, almost all known *A. dhakensis* virulence factors were highly upregulated *in vivo* including iron acquisition genes, the type 3 secretion system, Exotoxin A, and the lateral flagella. Pathway enrichment analysis revealed significant metabolic changes *in vivo* including upregulated amino acid synthesis, downregulated nucleotide and carbohydrate degradation, and an upregulation of glycolysis and fermentation but not respiration. Several genes were found to be upregulated *in vivo* which have never before been associated with *A. dhakensis* pathogenicity. To establish some of these as potentially novel virulence genes, 3 were chosen and in-frame deletion mutants along with *cis*-complemented strains were generated. *In vitro* and *in vivo* virulence was measured and found to be significantly attenuated compared to the parental strain to varying degrees.

GSMM 7

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

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Cystic Fibrosis (CF) is a genetic disorder characterized by chronic respiratory infections due to mutations in the CFTR gene, which lead to impaired chloride and bicarbonate transport in the lungs. These conditions create a unique environment that supports persistent infections, primarily caused by *Pseudomonas aeruginosa*. Over time, *P. aeruginosa* evolves resistance to antibiotics, limiting treatment options and contributing to high morbidity and mortality in CF patients. This study explores how CF lung conditions, particularly bicarbonate levels, influence the evolution of azithromycin (AZM) resistance in *P. aeruginosa*. Using adaptive laboratory evolution (ALE), we tracked the development of AZM resistance in the multidrug-resistant *P. aeruginosa* strain P4 under both bacteriological (CAMHB) and physiologically relevant (RPMI with bicarbonate) conditions. In CAMHB, *P. aeruginosa* rapidly evolved 128-fold resistance to AZM within 10 days, while in RPMI with bicarbonate, the bacteria surprisingly failed to develop resistance. Our previous studies suggest that bicarbonate, a critical component in CF lung physiology, plays a key role in modulating antibiotic susceptibilities. Further ALE experiments in CAMHB supplemented with bicarbonate revealed a critical finding: bicarbonate significantly interrupts AZM resistance evolution in *P. aeruginosa*, likely by blocking compensatory mutations responsible for developing AZM resistance. Ongoing work aims to identify the genetic mechanisms underlying this effect through whole-genome sequencing and extend these findings to other macrolide antibiotics and bacterial pathogens. This research highlights the influence of CF host factors, specifically bicarbonate, on the evolution of antibiotic resistance in *P. aeruginosa*, providing new insights that could guide therapeutic strategies in CF treatment.

GSMM 8

Exploring the mechanism of host immune-modulated esophageal gland-dependent schistosome development

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Schistosomes, parasitic flatworms and the causative agent of schistosomiasis, have a fascinating ability to neutralize leukocytes ingested from continuous blood feeding via the esophageal gland (EG), a digestive organ, essential for their survival. We ***hypothesize*** that the EG plays an important role in leukocyte-mediated parasite development. To better understand the nature of immune-modulated parasite development, we re-examined their growth in immunocompromised *Rag1*$^{-/-}$ mice. The onset of the developmental delay (~ 14 dpi) in *Rag1*$^{-/-}$ coincides with a stage when parasites begin to feed on blood, we investigated the role of EG-mediated blood feeding in host-dependent

parasite development. To do so, we fed whole blood cells (WBC) to larval schistosomes with or without the EG. Interestingly, while WBC feeding led to minor changes in the worm size and number of proliferative cells, the absence of the EG played a dominant role, in which EG-lacking larval were significantly smaller and had reduced number of proliferative cells. These results suggest a feeding independent developmental mechanism that requires a functional EG. Interestingly, RNA-seq of EG-lacking larvae identified 138 downregulated genes that were feeding independent and EG-dependent. In addition, our functional RNAi screen identified eight candidate genes that phenocopy EG-lacking (*foxA* RNAi) developmental defect. Our results demonstrate the crucial role of the EG in leukocyte-feeding-mediated cell-to-cell communication that regulate stem cell-driven parasite development. We expect to unveil potential druggable factors regulating developmental mechanisms deployed by these parasites. This work is supported by the Dean's startup fund, UT STAR award, and NIH/NIAID R01AI175079.

GSMM 9

MALDI-TOF MASS SPECTROMETRY AS A SURVEILLANCE TOOL OF MOSQUITO-BORNE VIRUSES

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Accurate and timely identification of mosquito species and infection status are necessary for surveillance programs to assess disease risk and monitor the spread of diseases. Current methods include PCR and morphological identification, but they can be expensive, time-consuming, and require specialized training. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has been proposed as a cheaper and quicker alternative. MALDI-TOF MS has been used to identify arthropods and human pathogens like *Plasmodium* spp., *Rickettsia* spp., *Bartonella* spp., and *Leishmania* strains in arthropods, but it has not been determined whether MALDI-TOF MS can also be used to detect arbovirus infection. This study examined the ability of MALDI-TOF MS to detect both mosquito species and infection status of *Aedes aegypti* and *Aedes albopictus* mosquitoes. Laboratory-reared uninfected and chikungunya-, dengue-, and Mayaro-infected mosquitoes were subjected to MALDI-TOF MS to generate protein profiles. Protein profiles were selected based on intensity and reproducibility to create a reference MALDI-TOF MS database. Mosquitoes of known infection status and species were blind tested against the database to assess the accuracy of detection. Our results indicate that MALDI-TOF MS can be used to differentiate mosquitoes of different species and infection status. Clarification is still required for whether MALDI-TOF MS can differentiate between the different viruses, but this approach appears to be able to distinguish alphaviruses from flaviviruses. The generation and sharing of the database with any scientist who has access to a mass spectrometer will aid in surveillance and outbreak response in endemic regions like Latin America.

GSMM 10

Low humidity enhances Zika virus infection and dissemination in Aedes aegypti mosquitoes

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As climate change alters Earth’s biomes, it is expected the transmission dynamics of mosquito-borne viruses will change. While the effects of temperature changes on mosquito-virus interactions and the spread of the pathogens have been elucidated over the last decade, the impact of relative humidity changes is still relatively unknown. To overcome this knowledge gap, we exposed Aedes aegypti females to various humidity conditions. We measured different components of vectorial capacity such as survival, blood-feeding rates, and changes in infection and dissemination of Zika virus. Survival decreased as the humidity level decreased, while infection rates increased as the humidity level decreased. Alternatively, blood feeding rates and disseminated infection rates peaked at the intermediate 50% relative humidity treatment but were the same in the 30% and 80% relative humidity treatments. These results provide empirical evidence that Ae. aegypti exposure to low humidity can enhance Zika virus infection in the mosquito, which has important implications in predicting how climate change will impact mosquito-borne viruses.

Graduate Students – General Microbiology

GSGM 1

***Trans*-translation inhibitors kill *Mycobacterium tuberculosis* and pathogenic non-tuberculous mycobacteria**

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With increased drug resistance and long treatment times, *Mycobacterium tuberculosis* and non-tuberculous mycobacteria are becoming difficult to treat. Antibiotics with new targets and novel mechanisms of action are need of the hour to combat drug resistant infections. The *trans*-translation ribosome rescue pathway is a potential target for anti-mycobacterial drugs because it is essential for survival in *Mycobacterium tuberculosis* (Mtb), and likely in non-tuberculous mycobacteria (NTM), but is not found in humans. *trans*-Translation is the primary mechanism to rescue ribosomes that are trapped at the 3′ end of mRNA. We have small molecules which inhibit *trans*-translation that kill Mtb and NTM. The most potent inhibitors are a group of oxadiazole ureas, which are bactericidal against Mtb and have minimum inhibitory concentrations (MIC) as low as 0.2 µg/mL. These molecules are orally bioavailable in mice and have advantageous pharmacokinetics. Structural and biochemical data showed that these inhibitors bind to the ribosome near the peptidyl-transfer center. Using an *in vitro trans*-translation assay made from purified Mtb ribosomes, Mtb translation factors, Mtb tmRNA, and Mtb SmpB, we showed that these compounds inhibit *trans*-translation with IC_{50} = 0.8 – 5.0 µg/mL but did not inhibit normal translation. These compounds were also bactericidal against *Mycobacterium avium* and *Mycobacterium abscessus*, with MICs 0.1 – 1.6 µg/mL. Some species specificity was observed, as the most potent compound was different for each mycobacterial species. Taken together, our findings suggest that *trans*-translation can be targeted to kill mycobacteria and inhibitors of *trans*-translation are candidates for future anti-mycobacterial drug development.

GSGM 2

Protein folding elements enable genetic innovation in antibiotic resistance genes

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Antibiotic resistance proteins enhance their activity through the rapid accumulation of mutations, leading to the emergence of bacterial strains that are no longer amenable to antibiotic treatment. Although powerful variants of resistance proteins have been subject to intense characterization, identification of general mechanisms controlling resistance evolution remains elusive. As a result, generation of strategies to mitigate the emergence of superbugs are currently non-existent. We have discovered that oxidative protein folding controls the expansion of the hydrolytic activity of β-lactamases, proteins that break down invaluable drugs like penicillin. We find that removal of conserved disulfide bonds from enzymes with narrow-hydrolytic spectra blocks their transformation into their broad-spectrum counterparts, while addition of disulfides in β-lactamases normally devoid of these linkages drastically expands their mutational landscapes.

We elucidate the molecular mechanism of evolution control and show that disulfide bonds act as anchors that facilitate the folding of evolved broad-spectrum enzymes, their presence offsetting the burden that functional mutations impose on protein biogenesis. Since disulfide formation is centrally catalyzed by the cell envelope oxidative pathway in bacteria, we demonstrate that targeting this process can limit genetic innovation in β-lactamases *in vitro* and *in vivo*, thus opening new avenues towards direly needed strategies that impede the evolution of antimicrobial resistance.

GSGM 3

Inhibition of Uropathogens by Boosting Toxicity of Endogenous Copper

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Urinary tract infections (UTI) are a major global health problem caused predominantly by uropathogenic E. coli (UPEC) and are the second leading cause of antibiotic use. Unfortunately, antibiotic resistance in UPEC and other uropathogens is climbing at an alarming rate, posing a threat to the current treatment practices. One novel approach is to exploit the nutritional immunity and its role in host-pathogen interactions with potential therapeutic agents. Copper, an effector of nutritional immunity, has been shown to be mobilized to the bladder as a host innate immune response to mitigate infection. We screened a comprehensive small molecule library in the presence of copper attempt to find novel inhibitory compounds of UPEC. The results of our high throughput screen revealed a molecule (ECIN) with bactericidal activity that works synergistically with copper against UPEC and other uropathogens. To begin evaluating the therapeutic potential of this compound, we have tested and detected modest-to-no cytotoxic effect of ECIN against human bladder epithelial cells and hepatocytes. Additionally, we have shown *in vitro* that ECIN kills UPEC adherent to bladder epithelial cells. To further our translational assessment of ECIN, we have collected cytotoxicity studies on porcine bladders exposed to various concentrations of ECIN. Ongoing and future experiments for a localized treatment of ECIN include infecting and eradicating UTI *in vivo* using both the murine and porcine

model of UTI. In summary, we have identified a novel small molecule that kills uropathogens by augmenting the toxicity of host-derived immune effector.

GSGM 4

Understanding The Abiotic Impacts of Land Development on Soil Micro diversity

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Land development and industrialization cause subsequent abiotic factors that influence microbial diversity and their antibiotic-producing properties. The aim of this research is to investigate the effect land development has on the soil that harbors these microbes and help develop strategies to preserve and protect these essential microorganisms. We are using 16S rRNA gene sequencing to determine microbial diversity while EPA Method 3050B and 1340 will detect any metal and lead contaminations, soil types will also be considered as potential sources of variance. VIDA is a 600-acre housing development adjacent to Texas A&M San Antonio University, which utilizes Low Impact Development (LID) in an effort to preserve surrounding ecosystems. Soil samples have been collected over the duration of a year during land development in VIDA. LID preservation includes the construction of a greenway and greenway sample sites will be compared to Residential and Undisturbed sample sites. We hypothesize that greenway sites will more closely resemble the microbiome of undisturbed sites given that LID practices effectively preserve surrounding ecosystems and will have less metal contamination. The efficacy of LID's ability to preserve microbial ecosystems will be evaluated using microbial diversity and the abundance of microbes producing antimicrobial properties. Analysis of chemical and soil composition will provide an understanding of what abiotic factors drive diversity across sample sites.

GSGM 5

Fungi of the order Mucorales express a “sealing-only” tRNA ligase

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Some eukaryotic pre-tRNAs contain an intron that is removed by a dedicated set of enzymes. Intron-containing pre-tRNAs are cleaved bytRNA splicing endonuclease, followed by ligation of the two exons and release of the intron. Fungi use a “heal and seal” pathway that requires three distinct catalytic domains of the tRNA ligase enzyme, Trl1. In contrast, humans use a “direct ligation” pathway carried out by RTCB, an enzyme completely unrelated to Trl1. Because of these mechanistic differences, Trl1 has been proposed as a promising drug target for fungal infections. To validate Trl1 as a broad-spectrum drug target, we show that fungi from three different phyla contain Trl1 orthologs with all three domains. This includes the major invasive human fungal pathogens, and these proteins can each functionally replace yeast Trl1. In contrast, species from the order Mucorales, including the pathogens *Rhizopus arrhizus* and *Mucor circinelloides*, have an atypical Trl1 that contains the sealing domain but lacks both healing domains. Although these species contain fewer tRNA introns than other pathogenic
fungi, they still require splicing to decode three of the 61 sense codons. These sealing-only Trl1 orthologs can functionally complement defects in the corresponding domain of yeast Trl1 and use a conserved catalytic lysine residue. We conclude that Mucorales use a sealing-only enzyme together with unidentified nonorthologous healing enzymes for their heal and seal pathway. This implies that drugs that target the sealing activity are more likely to be broader-spectrum antifungals than drugs that target the healing domains.

GSGM 6

Transcriptomic analysis of a temperature-dependent regulatory protein in *Pseudomonas aeruginosa* biofilms

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As bacteria transition from one niche to another, they must adapt to external stimuli such as temperature. One such microbe is *Pseudomonas aeruginosa,* which is known to form robust biofilms that are significantly more tolerant to antibiotics and immune cells than its planktonic counterpart. Previously our lab group observed that *P. aeruginosa* biofilm extracellular polymeric substance (EPS) changes in response to temperature, and we identified some genes essential for biofilm formation in specific temperature ranges. We now seek to further characterize the temperature-dependent mechanisms associated with these biofilm adaptations. To answer these questions, we conducted an

RNAseq analysis of *P. aeruginosa* PA14 biofilms after 48-hour growth period at four different temperatures: 23˚, 30˚, 37˚ and 40˚C. The mRNA from the biofilms and the remaining planktonic cells were extracted and sequenced, and differential expression was evaluated.
Through this analysis, we were able to identify a regulatory protein which was differentially expressed in biofilms in response to external temperature. Specifically, an uncharacterized regulator, PA14_26330, was found to be upregulated in biofilms at environmentally-relevant temperatures. We then conducted an RNAseq analysis on a mutant lacking PA14_26330 to identify the specific temperature-responsive regulons on this protein. In our work so far, we believe that PA14_26330 may be a temperature-dependent global response regulator that effects processes that we have previously found to have temperature-specific phenotypes such as iron homeostasis, oxidative phosphorylation and the attachment pili.

GSGM 7

The whodunit of poop: Microbial source tracking uncovers the drivers of fecal pollution in Baffin Bay, Texas.

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Fecal pollution is a leading cause of water impairment along the Texas coast. Pathogenic microorganisms in fecal pollution can cause harmful infections in people who recreate in impaired waters. Furthermore, residual pharmaceuticals and chemical compounds present in fecal pollution can damage natural ecosystems. Baffin Bay is an enclosed, hypersaline bay in a predominantly rural watershed on the Texas coast. Three of the major tributaries feeding into Baffin Bay have segments that are classified as impaired due to high levels of fecal indicator bacteria (FIB). This study conducted a comprehensive water quality assessment, including microbial source tracking (MST), to determine the sources and drivers of fecal pollution. Eighteen monthly sampling events (n=12 sites, n=216 samples) revealed that traditional FIB consistently exceeded the USEPA's recommended threshold, particularly in the impaired tributaries. Findings also demonstrated that cow (mean=2,428.0 gene copies/100mL), pig (mean=938.8 gene copies/100mL), and human (mean=304.2 gene copies/100mL) fecal markers were nearly omnipresent within the tributaries, whereas the gull fecal marker was highest in the bay (mean=703.8 gene copies/100mL). The cow marker's widespread presence in the bay (88% of samples) and tributaries (96% of samples) peaked at 71,540 gene copies/100mL in San Fernando Creek, indicating that cattle are a significant contributor of fecal pollution. These findings underscore the need for mitigation strategies and watershed management decisions that incorporate source-specific MST data.

GSGM 8

Role of GPI8 in Regulating Surface Molecules, Toxin Resistance, and Virulence in *L. major* : Insights from GPI Anchor Deficient Mutants

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Glycosylphosphatidylinositol (GPI) anchoring of various surface proteins contributes to survival, pathogenicity, and immune evasion in *Leishmania*. During the infection cycle, *Leishmania* may encounter bacteria like *Aeromonas spp.*, producing pore-forming toxins (PFTs) like aerolysin, which bind to GPI-anchored proteins. Studying PFTs provides insights into membrane repair mechanisms and parasite resilience in host gut. GPI8 is an important catalytic subunit of the GPI transamidase complex that adds GPI anchors to newly synthesized proteins like lipophosphoglycan (LPG) and gp63. Previously, GPI8 knockout mutants in *L. mexicana* showed normal growth, differentiation, and infectivity, suggesting that GPI-anchored proteins are not essential for growth, survival, and virulence in this species. In this study, we generated tagged and knockout mutants of GPI8 in *L. major*. The mutant serves as a global knockout as it hinders the expression of all GPI-anchored proteins, including the major virulence factor gp63. *L. major* knockout mutants exhibit significant growth defects, characterized by slower growth and metacyclogenesis rates than wild-type. In addition, we pioneered the report that aerolysin kills wild-type promastigotes, whereas lacking GPI8 makes it resistant to toxin treatment. Interestingly, this protection is dependent on increased levels of LPG, which may prevent aerolysin binding to other GPI-anchored molecules. Increased expression of LPG in the mutants could potentially enhance virulence in mouse models. These contrasting results highlight species-specific differences in the role of GPI-anchored proteins in parasite biology and virulence. This study highlights the complex role of GPI8 in regulating *Leishmania* surface molecules, with implications for understanding parasite biology and potential therapeutic targets.

GSGM 9

Exploring and exploiting synergy between macrolides and tetracyclines against *A. baumannii*

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Finding novel approaches to combat bacterial growth becomes more critical as antibiotic resistance rises. The exploitation of synergistic antibiotic combinations, however, is an exciting potential solution. Our prior work found synergy between the tetracycline minocycline (MIN) and the macrolide azithromycin (AZM) against A. baumannii. Although found to be highly effective both in vitro and in vivo, the mechanistic basis for synergy and the optimal therapeutic usage against A. baumannii was unclear. To clarify the mechanism, we conducted fractional inhibitory concentration (FIC) assays with AZM and MIN against four different strains of A. baumannii in two different media (CA-MHB and RPMI+). Synergism between AZM and MIN was detected against every strain in each media, indicating this interaction is strain- and media-independent. We next sought to determine if this synergy was specific to AZM and MIN or a broad phenomenon with tetracyclines and macrolides. Upon further FIC testing with other antibiotics of each class, it was found that only AZM, in combination with any tetracycline, displayed synergy, whereas all other non-AZM macrolide combinations displayed additivity. We hypothesized the essential role of AZM in these synergistic combinations was due to its enhanced kinetics of translation inhibition. Using our established translation assays, we found that while there were small differences between the tetracyclines, AZM was a much more potent translational inhibitor against A. baumannii compared to the other macrolides. These findings suggest a kinetic-dependent mechanism behind tetracycline and macrolide synergy, which can be used to improve treatment against the increasingly drug-resistant A. baumannii.

GSGM 10

Comparison of MALDI-TOF MS and Whole Genome Sequencing for Identification Vibrio parahaemolyticus Strains Isolated from Oysters

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Early identification of *V. parahaemolyticus* is crucial for ensuring food safety and for monitoring and predicting outbreaks. However, whole genome sequencing (WGS) - the current standard for bacterial phylogeny - is costly and labor-intensive. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) offers a rapid, accurate, and cost-effective method for bacterial identification based on polypeptide analysis; however, few studies have compared the resolution of MALDI-TOF MS with WGS for *V. parahaemolyticus*. In preliminary work, 67 bacteria were isolated on differential and selective media from wild oysters collected in Galveston Bay, an aquaculture facility in Massachusetts, and from seafood markets in Kemah, TX. All isolates were identified as *V. parahaemolyticus* using MALDI-TOF MS, which agreed with identification based on WGS. Cluster analysis of spectra generated by MALDI-TOF MS revealed three distinct clusters that corresponded to the source of the oysters. WGS of the 23 isolates purchased from seafood market oysters, yielded a phylogenetic tree with eight clusters and showed that the average nucleotide identity between these strains exceeded 98%. Notably, two clusters from the phylogenetic tree and the MALDI-TOF dendrogram were identical. These results suggest that MALDI-TOF MS can differentiate the source of *Vibrio parahaemolyticus* strains isolated from oysters and has a resolution comparable to WGS.

GSGM 11

***Ehrlichia chaffeensis* TRP120-mediated NFAT signaling and chemokine expression**

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Ehrlichia chaffeensis (*E. chaffeensis*) is a tick-transmitted, obligately intracellular, gram-negative bacterium responsible for the life-threatening zoonoses, human monocytic ehrlichiosis (HME). *E. chaffeensis* exhibits tropism for mononuclear phagocytes and has evolved distinct survival strategies to evade immune defenses by secreting effector proteins that reprogram the host cell. TRP120 (120-kDA tandem repeat protein) is a key effector with multiple functions that promote infection and transmission. Our laboratory's initial findings suggest that LPS-deficient *E. chaffeensis* stimulates the secretion of numerous chemokines, likely through activation of cellular signaling pathways using novel short linear motif (SLiM) ligand mimicry. Various SLiMs that mimic endogenous ligands are found within the intrinsically disordered tandem repeat (TR) regions of TRP120 and have been shown to activate evolutionarily conserved cellular pathways such as Wnt, Notch, Hedgehog, and Hippo. Interestingly, we

have determined that the non-canonical Wnt transcription factor, NFATc1 (nuclear factor of activated T cells), is activated by TRP120 and that NFATc1 inhibition results in a significant reduction in monocyte chemoattractant protein 1 (MCP-1) during infection. We hypothesize that *E. chaffeensis* TRP120, acting as a Wnt ligand mimic, activates NFAT signaling thereby promoting chemokine expression and monocyte recruitment. NFAT signaling plays a crucial role in the function and development of innate myeloid cells and regulates the expression of potent immunomodulatory chemokines. Utilizing molecular approaches such as confocal microscopy, immunoblot analysis, ELISA assays, and RT^2 PCR arrays, our objective is to elucidate the mechanism of NFAT activation and its role in facilitating a pathogen beneficial response during *E. chaffeensis* infection.

Thematic Faculty and Trainee Oral Presentations

Vector-borne diseases & Parasitology

VP-1T

Comparative Genomics of Soft Tick-borne Relapsing Fever Spirochete Genomes Yields New Insights into Plasmid Diversity and Antigenic Variation Systems

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Tick-borne relapsing fever (TBRF) is a globally prevalent, yet under-studied vector-borne disease transmitted by soft and hard bodied ticks. *Borrelia* spirochetes, causative agents of Lyme disease and relapsing fever (RF), have uniquely complex genomes, consisting of a linear chromosome and both circular and linear plasmids. In Lyme spirochetes, plasmid-borne genes play an essential role in the vector-host life cycle; however, this is not well understood in RF *Borrelia* due to the lack of plasmid-resolved genome assemblies. Using third-generation sequencing technology coupled with a *Borrelia*-specific genome assembly pipeline, we were able to overcome previous hurtles in RF spirochete genomics. We generated high quality, plasmid-resolved genome assemblies for seven RF species found in the Western Hemisphere. Comparative genomics analysis indicated substantial diversity in the plasmids clustering them into 30 plasmid families. Analysis of the antigenic variation systems, a critical pathogenic feature for immune evasion, highlighted mechanistic differences as well as differences in gene content related to this system. Interestingly, in *Borrelia hermsii* the sole expression site for antigenic variation was found on the F20 plasmid family; whereas, in the other species we investigated (except for *Borrelia anserina*) this site was on the F28 plasmid family. *Borrelia hermsii* has been the model for antigenic variation in RF spirochetes, therefore our findings highlight the need to expand our mechanistic understanding of this system to include other RF spirochete species. Collectively, this analysis provides the foundation for future investigations to identify essential genetic elements that drive the vector-host life cycle of RF *Borrelia*.

VP-2T

Multidimensional approach for the assessment of the risk of *Ehrlichia canis* infection in Iquitos, Peru

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In Iquitos, the high prevalence of *Rhipicephalus sanguineus* s.l. ticks may increase the risk of tick-borne diseases (TBDs) for both dogs and their owners, with *Ehrlichia canis* being particularly concerning due to its severity in dogs

and zoonotic potential. We conducted a cross-sectional study with three objectives: 1) assess *E. canis* prevalence in dogs, 2) identify factors associated with infection, and 3) examine whether dog owners' perception of tick-bite risk and TBD influences their use of preventive measures. Blood samples were collected from 388 dogs in 285 randomly selected households across Iquitos. Two trained field workers administered a questionnaire to gather data on potential risk factors, including owners' socio-demographics, housing characteristics, and dog-related information. Additional data on risk perception and tick prevention practices were also collected. All blood samples were processed by real-time PCR (qPCR) targeting the *E. canis* dsb gene. We used multivariate mixed effects logistic regression models to evaluate the association between risk factors and *E. canis* qPCR positivity. The final model was built using a manual forward nested approach based on the Akaike Information Criterion (AIC). The prevalence of *E. canis* among dogs was 19.6% (95% CI 15.8 – 23.9%). Our study sheds light on control strategies that could improve the problem. In this study setting, intervention strategies for the prevention and control of *E. canis* may benefit from prioritizing dogs living in homes with corrugated iron walls. Comprehensive interventions, including targeted campaigns for dogs that have recently traveled, as well as neutering/spaying programs, could be beneficial.

VP-3T

Reduced microbe abundance in an urban larval development container increases *Aedes aegypti* susceptibility to Zika virus

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Aedes aegypti mosquitoes are a major vector of arboviruses that oviposit in both artificial containers (i.e. buckets, tires, cans) and natural containers (i.e. coconut husks, tree holes). These diverse container types will seed the larvae microbiome with differing bacterial communities. While the larval microbiome has been shown to alter adult susceptibility to arboviruses including dengue (DENV) and Zika virus (ZIKV), it is not known if exposure to different bacterial communities found between container types impacts adult *Ae. aegypti* interactions with arboviruses. To address this, rainwater was collected from an artificial container (plastic buckets) and a natural container (coconut husks) from three different collection sites and the microbiomes were preserved. Larval exposure to plastic bucket-derived microbiomes resulted in adults with increased susceptibility to ZIKV compared to larval exposure to coconut husk-derived microbiomes from all three collection sites, indicating that the container type, independent of collection environment, drives variation in adult susceptibility to ZIKV. 16S amplicon sequencing of larvae exposed to the preserved microbiomes revealed that bacterial community structure differed between plastic bucket and coconut husk derived communities at each collection site, but a conserved plastic- or coconut-derived bacterial community across collection sites was not identified. However, water from coconut husks had significantly more total bacterial abundance than water from plastic buckets. Normalization of bacterial loads between container types resulted in similar ZIKV infection rates. Together, these data suggest that larval exposure to specific container type-associated microbiomes alters adult susceptibility to ZIKV, largely driven by differences in total bacterial density between container types. Results from this study will help understand how the urbanization-driven expansion of *Ae. aegypti* into new/different oviposition sites might affect arbovirus susceptibility.

VP-4T

Role of Morrbid-3 lncRNA in signaling macrophage proinflammatory response for control of *Trypanosoma cruzi* infection

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Trypanosoma cruzi (Tc), the causative agent of Chagas cardiomyopathy, is widely distributed in Latin America and the southern half of the USA. Macrophages (Mφ) sub-par activation of immune responses to *Tc* is suggested to contribute to parasite dissemination and persistence; though the mechanisms by which *Tc* hijack the innate immune

system to ensure its survival in the host are largely unknown. Long non-coding RNAs (lncRNAs, > 200 nucleotides) include a diverse class of RNAs that do not encode proteins. Among them, a class of lncRNA that binds to RNA binding protein (RBP) is regarded to play a critical role in regulation of post-transcriptional / translational machinery and protein synthesis. Whether lncRNA-RBP interactions shape host immunity remains an underexplored opportunity for control of parasitic infection. Through systematic analysis of lncRNA arrays and RTqPCR verification, we identified transcript variants 1-3 of lncRNA Morrbid were upregulated in cultured and primary Mφ infected with *Tc*. SiRNA and GapmeR were used to deplete Morrbid-3 expression in infected Mφ. Additional data showed that Morrbid-3 depletion intensified the proinflammatory cytokines/chemokines gene expression and offered significant control of intracellular parasites in Mφ. Unbiased screening of cross-linking immunoprecipitation-sequencing databases and subsequent RNA immunoprecipitation (RIP) assays demonstrated that lncRNA Morrbid-3 binds to three RBPs that have a function in regulating the innate immune response. We are currently performing mechanistic studies to explore the Morrbid-3/RBPs mediated immune regulation of Mφ with an aim to arrest parasite survival and replication.

Antimicrobial Resistance & Microbiome Research

ARM-1F

Structural bases of bacterial persistence and antibiotic resistance

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During starvation and stress, many organisms use hibernation factor proteins to inhibit protein synthesis and protect their ribosomes from damage. While several ribosome hibernation promoting factors have been described in the model bacterium *Escherichia coli*, the mechanisms of translation shutdown in slow growing bacteria such as *Mycobacterium tuberculosis* remain elusive. Regulation of the activity of ribosomes in *M. tuberculosis* under hypoxic and nutrient starvation conditions has emerged as an important aspect of *M. tuberculosis* biology and persistence. Combining cryo-electron microscopy (cryo-EM) and mass-spectrometry, we identified a new ribosome hibernation factor in slow growing bacteria that silences actively translating ribosomes, which differs from the well-known ribosome hibernation mechanism in fast growing bacteria such as *E. coli*. Furthermore, several of the ribosome hibernation protein factors bind to ribosomal sites that overlap with those of antibiotics, providing insights into resistance and persistence of dormant *M. tuberculosis*. In collaboration with several groups, we are developing approaches to visualize at high-resolution ribosomes isolated from stressed pathogenic bacteria.

ARM-2T

The natural reservoirs of Mobile Colistin Resistance proteins

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Colistin is a crucial last resort antibiotic used to treat bacteria that are resistant to all other antimicrobials. Unfortunately, increased use of colistin has led to the emergence of resistant strains. This surge in resistance is, in part, due to novel Mobile Colistin Resistance (MCR) proteins which modify the lipid A of Gram-negative bacteria and prevent colistin entry into the cell. MCR genes are mobilizable and transfer easily between different bacteria and their rapid spread across the globe constitutes a serious threat to the efficacy of colistin. With only a few representative MCR sequences characterized to date, the diversity of this protein superfamily is greatly understudied. This along with the inherently unreliable nature of phenotypic colistin susceptibility assays, makes detection, surveillance, and treatment of MCR-carrying strains challenging.

We have discovered that *mcr*-like sequences are commonly present in Proteobacteria, including in many species that do not cause disease. Our bioinformatic analysis unveiled that ~40% of these *mcr*-like elements harbor clear signatures of horizontal gene transfer. Through expression of a diverse set of *mcr*-like genes in clinically relevant bacteria like *Escherichia coli* or *Enterobacter cloacae*, we identified several novel enzyme classes that can readily confer colistin resistance. Our results suggest that environmental Proteobacteria act as a rich reservoir of new classes of *mcr* genes that, if transferred to the clinic, would further contribute to the surge of colistin resistance. Expanding

our understanding of this new MCR sequence space is crucial to laying the groundwork for improved detection of novel high-risk MCR proteins.

ARM-3F

Iron Memory in *E. coli*

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Constantly changing environments present a challenge to the survival of all organisms. They can adapt to change either by behavioral or phenotypic plasticity but must decide among available phenotype choices. Vertebrates use their nervous system for faster decision-making by storing information about their prior experiences. The process of storage and retrieval of information is called memory. The importance of memory in bacterial decision-making is relatively unexplored. We report here a multigenerational memory in *Escherichia coli* swarming motility, where cells 'remember' their swarming experience for several generations – a prior experience of swarming improving its future swarming efficiency. We performed over 10,000 single-cell swarm assays and discovered that cells store memory as cellular iron levels. This 'iron' memory exists in planktonic cells but the act of swarming reinforces it. A cell with low iron swarms early and more efficiently, while cells with high iron do the opposite. Swarming potential of a mother cell, which tracks with its iron memory, is inherited up to the fourth generation but is naturally lost by the seventh. Artificially manipulating iron levels prolongs this memory. A mathematical model with a time-delay accurately captures these dynamics. We also found that cellular iron levels correlate with biofilm formation and antibiotic tolerance, suggesting broader physiological implications.

ARM-4T

Genetic Basis of Cefiderocol Nonsuceptibility in Pseudomonas aeruginosa

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Treating *Pseudomonas aeruginosa* infections is becoming increasingly difficult due to the development of antimicrobial resistance via intrinsic (porin loss, multidrug efflux) and acquired mechanisms (resistance genes on mobile genetic elements). Cefiderocol is a recently FDA-approved cephalosporin antibiotic that possesses a chlorocatechol siderophore moiety that allows it to chelate ferric iron and efficiently enter the pathogen via iron-uptake receptors. It is also purported to be an inefficient substrate for drug efflux and stable against most β-lactamases. However, there are increasing reports of emergence of resistance on therapy.

To identify genetic factors associated with cefiderocol resistance, we screened ~200 carbapenem-resistant clinical isolates for decreased cefiderocol susceptibility by broth microdilution testing (MIC ≥ 8μg/mL) and population analysis profile (bacterial subpopulation growing at ≥ 8μg/mL on agar dilution). We identified 6% of isolates that were nonsusceptible to cefiderocol and a further 14% displaying heteroresistance despite no prior drug exposure. These isolates were more likely to harbor previously-identified mutations in iron-uptake genes that either inactivate or downregulate cefiderocol import pathways. We more thoroughly examined five closely-related isolates consisting of one susceptible and four nonsusceptible or heteroresistant strains. Here, resistance was attributed to the production of β-lactamases (CTX-M, KPC, VIM) or hypermutability (inactivation of missense repair protein MutS). For β-lactamase-producing isolates, the broad-spectrum inhibitor taniborbactam sensitized the pathogen to cefiderocol, resulting in an 8-16-fold decrease in the MIC. For isolates harboring *mutS* mutations, cefiderocol selected for a stably-resistant subpopulation. Understanding the mechanisms of cefiderocol resistance in these isolates can reveal therapeutic strategies to preserve the utility the drug.

ARM-5T

Imipenem-relebactam to restore efficacy of first-line drugs and develop novel combination regimens to treat multidrug-resistant tuberculosis

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Background. We tested imipenem with and without a b-lactamase inhibitor, relebactam, (i) to determine minimum inhibitory concentration (MIC), (ii) to perform static concentration-kill (STK) studies, and (iii) rank various combinations with isoniazid, rifampin, moxifloxacin, tedizolid, pretonamid, bedaquiline, and omadacycline to identify backbone regimens with activity against MDR-TB.

Methods. An MDR-TB clinical strain (SAMRC-16D) was used in all experiments. MIC and STK studies were performed using the Mycobacterial growth indicator tube system. The concentrations of the drugs used in experiments were those achieved with standard doses of each drug in the treatment of TB.

Results. The MICs of imipenem alone or in combination of relebactam 32mg/L and 2mg/L, respectively. MICs (mg/L) of isoniazid, rifampin, moxifloxacin, tedizolid, pretonamid, bedaquiline, and omadacycline were 1, 32, 0.125, 0.25, 0.125, 0.25, and 2, respectively. Imipenem maximum kill (E_{max}) was 3.21±0.19 $\log_{10}$ CFU/mL with concentration associated with 50% of the maximal effect (EC_{50}) as 28.88±3.08 mg/L (r^2=0.99). Relebactam improved both efficacy (E_{max} = 4.89±0.47 $\log_{10}$ CFU/mL) and potency (EC_{50} = 3.37±1.14 mg/L) of imipenem (r^2=0.97). Isoniazid, rifampicin, moxifloxacin, tedizolid, and pretonamid, when combined with imipenem-relebactam killed 4.97±0.01 $\log_{10}$ CFU/mL, whereas kill with bedaquiline and omadacycline combinations was 3.12 $\log_{10}$CFU/mL and 3.97 $\log_{10}$ CFU/mL, respectively.

Conclusion. Relebactam improved the imipenem MIC, efficacy, and potency by multiple folds. Imipenem-relebactam combination restored the efficacy and potency of isoniazid and rifampin evidenced by the killing of MDR-TB. The backbone regimens identified here need further evaluation at dynamic concentrations.

ARM-6F

More Than Just a Buffer: Bicarbonate Dictates Antibiotic Activities, Susceptibilities, and Resistance

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Accurate prediction of antibiotic susceptibility *in vitro* is a cornerstone of modern infectious disease care. However, current antimicrobial susceptibility testing often fails to accurately estimate the efficacy of antibiotics in patients as the standardized bacteriologic medium used is not reflective of host conditions. Antimicrobial susceptibility testing in a more physiologically relevant medium has previously permitted the discovery of antibiotics with unrealized activity against multidrug-resistant (MDR) pathogens. Expanding upon these findings we have sought to identify antibiotics impacted by host conditions and define the mechanistic basis for their altered activities. We have uncovered differential responses to physiologically relevant media spanning within and between antibiotic classes. While some classes are not affected, macrolides, tetracyclines, and fluoroquinolones are impacted both positively and surprisingly, negatively. Contrasting the composition of the two media led to the identification of the key component dictating the observed differential antibiotic activities, the biological buffer bicarbonate. We've now found that bicarbonate, independent of its role in regulating pH, impacts how antibiotics work against multiple MDR pathogens, alters host-pathogen interactions, and even controls how bacteria evolve antibiotic resistance. Elucidating how the small chemical compound bicarbonate plays such a big role in infectious disease treatment will aid in our understanding of how host conditions impact antibiotic activities, and may pave the way for future host modulatory treatment approaches.

Environmental Microbiology

EM-1F

Microbial eukaryotic contributions to the food web at deep-sea hydrothermal vents

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Deep-sea hydrothermal vents are oases of microbial and animal biological diversity that is made possible by primary production sourced by chemolithoautotrophic microorganisms. Yet, the rate and route of how this energy is transferred to higher trophic levels to sustain the local hydrothermal vent community remains understudied. This research addresses the ecological contributions that single-celled microbial eukaryotes (protists or microeukaryotes) make to

deep-sea food webs, with a particular focus on microbial mortality (e.g., grazing, parasitism). Centered at two vent fields at the Mid-Cayman Rise, Von Damm and Piccard, we quantified protistan predation pressure (grazing on bacteria and archaea) and biomass (derived from cell abundance) to more clearly understand the flow of carbon through the hydrothermal vent food web. Protists were found to exert predation pressure on the local hydrothermal vent bacteria and archaea, which may account for the trophic transfer of a substantial amount of carbon. We also used genetic methods to link transcript profiles to microeukaryotic activities, specifically identifying metabolic pathways associated with heterotrophic modes of nutrition. Results from meta-omic analyses highlight how trends in expressed transcripts clustered similarly to taxonomic diversity across the vent sites and provide new insight into generalist vs. specialist lifestyles among vent-associated protists. Altogether, our study contributes to a clearer understanding of how microeukaryotic species mediate linkages between the hydrothermal vent food web to the broader deep-sea carbon budget.

EM-2F

Soil microbial community responses to long-term environmental disturbances

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Long-term and chronic environmental disturbances such as nutrient enrichment and oil/heavy metal pollution can alter the structure and functions of rhizosphere soil microbial communities (soil microbes that are in direct contact with plant roots), shifting their trajectories over time. Microbial communities, however, might have the ability to bounce back or adapt, depending on the community composition or microbial members, the type and degree of disturbances and the prevailing environmental conditions. We explore how different environmental disturbances impact and/or influence microbial communities and their interactions with plants in coastal ecosystems. We examine these responses across different systems, spanning observational and field experiments, to draw comparisons/contrast and patterns of microbial responses. For example, long-term exposure to heavy metal and oil contaminants can lead to a highly distinct communities (in taxonomic composition and functional potential). In contrast, repeated and prolonged exposure to nutrient enrichment may not necessarily lead to altered rhizosphere community structure. Insights gained from these different studies enrich fundamental knowledge on microbial community dynamics, including how disturbances alter microbial interactions, and better prediction on how microbe-mediated soil processes will change with environmental disturbances. Such knowledge can also potentially inform or aid coastal resource management.

EM-3T

High Prevalence of Avian Influenza Viruses in Vietnam's Live Bird Markets

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Live Bird Markets (LBMs) have been the site of numerous human infections with avian influenza virus (AIV). We conducted surveillance for novel AIVs at LBMs in northern Vietnam. During the period of August 2021 to August 2023, a total of 688 samples including 354 poultry worker human nasal washes (NW), 67 poultry orotracheal swabs (OS), 90 fecal cage swabs (FS) and 177 bioaerosol samples (BioS) were collected at LBMs in Hanoi city and three northern Vietnam provinces of Lang Son, Lao Cai and Quang Ninh which have border with China. From these samples, 6 (1.6%) NW, 59 (88.1%) OS, 51 (56.6%) FC and 5 (2.8%) BioS had molecular evidence of AIV (n=121, 17.5% overall). Highly Pathogenic AIV subtype H5 was detected in 4 (1.1%) NW, 8 (11.9%) OS and 12 (13.3%) FS samples. All the H5+ NW specimens, 3 (3.3%) OS and 4 (4.4%) FS specimens had molecular evidence of concomitant H9 AIV, further characterized as H9N2. In 17 (18.8%) OS samples, a H6N2 virus was detected. Similar prevalences of viral detections were found at each LBM site. However, positive bioaerosol samples came only from the most poultry-dense LBM which was located near Hanoi city. We conclude that LBMs in northern Vietnam have a high prevalence of AIVs which have potential to spill over to infect humans.

EM-4T

Cyanotoxin negatively associates with microbial functional redundancy in lakes of the southcentral USA

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Globally, warming of surface waters with climate change and eutrophication have led to an increased frequency and magnitude of harmful algal blooms (HAB). It has been surmised that the stability of aquatic ecosystems is linked to the redundancy of ecosystem functions, and that resilience can be disrupted by harmful algal blooms. In this study we analyzed total microcystin concentrations, functional redundancy and functional abundance related to the nitrogen and phosphorus cycles. The scope of the sampling comprised twenty eutrophic lakes distributed across a strong east-west precipitation gradient, sampled seasonally (spring and summer) in deep and shallow water stations. The functions were identified in metagenomic data. In the spring samples, the functional redundancy of nitrogen fixation, photosynthesis, nitrogen and phosphonate metabolism, oxidative phosphorylation, organic phosphate metabolism, and protein phosphorylation were low, while total microcystin concentrations were high. A negative association between total microcystin concentrations and the redundancy in phosphonate transport and organic phosphonate metabolism was notably observed as a seasonal trend. This suggests that among the many functional redundancies negatively impacted by microcystin , phosphonate cycle redundancy was the most fragile. Future studies are necessary to determine how microcystin concentration affects the availability of phosphorus. It may be that cyanobacteria blooms, while promoting the accumulation of nitrogen, also increase the accessibility of phosphorus.

EM-5F

Do Cnidarians remember previous foe attacks?

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Cnidarians, in general, are long-lived organisms and hence may repeatedly encounter common pathogens during their lifespans. It remains unknown whether these early diverging animals possess some type of immunological reaction that strengthens the defense response upon repeated infections, such as that described in more evolutionary derived organisms. Here we show results that in the sea anemone, *Exaiptasia diaphana* that had previously encountered a pathogen under sub-lethal conditions had a higher survivorship during a subsequently lethal challenge than naïve anemones that encountered the pathogen for the first time using two different known coral pathogens. *E. diaphana* subjected to the lethal challenge two and four weeks after the sub-lethal exposure using *Vibrio coralliilyticus* presented seven-(p=0.031) and five-fold (p=0.039) increases in survival, respectively, compared to the naïve anemones. However, anemones challenged six weeks after the sub-lethal exposure showed no increase in survivorship. *Serratia marcescens* challenged anemones subjected to a lethal exposure four weeks after the sub-lethal exposure showed increased survivorship with 25% surviving the secondary exposure (p=0.000). This short-lasting priming of the defense response could be ecologically relevant if pathogen encounters are restricted to short seasons characterized by high stress. Further studies are needed to determine if priming is pathogen specific as *V. coralliilyticus*appears to provide specificity wile *S. marcescens* does not. The ability to confer specificity may be dependent on how common the pathogen is in the environment. These findings reveal that immunological priming may have evolved much earlier in the tree of life than previously thought.

EM-6T

Roles of Two Extracytoplasmic Function Sigma Factors and a Signal Peptide in Symbiotic Nitrogen Fixation and Desiccation Stress Response of *Bradyrhizobium japonicum*.

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The soil bacterium *Bradyrhizobium japonicum* forms a symbiotic relationship with soybeans (*Glycine max*), fixing atmospheric nitrogen into ammonia, which is essential for sustainable agriculture. However, drought conditions can severely inhibit this process by reducing rhizobial survival. To enhance bacterial resilience in arid soils, we investigated the roles of two extracytoplasmic function (ECF) sigma factors, Bll2628/PrtI and Bll3014, and the potential signal peptide Blr5636, which contains a FecR domain and interacts with these ECFs. Using site-specific mutagenesis, we generated knockout mutants to assess their desiccation tolerance and symbiotic efficiency. In desiccation assays, *bll2628* and *bll3014* mutants showed significantly reduced survival compared to the wild type, while *blr5636* mutants initially exhibited lower survival but improved after 72 h. Trehalose production, a key factor in desiccation tolerance, was reduced in *bll2628* and *bll3014* mutants but unaffected in the *blr5636* mutant. In symbiosis experiments, plants inoculated with mutant strains exhibited reduced nitrogen fixation, with *bll3014* and *blr5636* mutants showing particularly low nitrogenase activity compared to the wild type. qRT-PCR analysis revealed that deletion of *blr5636* affected the expression of *bll2628* and *bll3014* during the early stage of desiccation, while gene complementation influenced *aceA* gene regulation during both the early and late stages of stress. These findings highlight the critical roles of Bll2628, Bll3014, and Blr5636 in desiccation tolerance and nitrogen

fixation, providing potential gene targets to improve *B. japonicum* survival and symbiotic efficiency in drought-prone environments.

EM-7F

Interactions between cyanotoxin producers and degraders in twenty lakes across southcentral USA

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Nutrients imbalances (particularly concerning nitrogen and phosphorus) caused by agricultural and wastewater runoffs into freshwater bodies often lead to the proliferation of cyanobacterial harmful algal blooms (cHABs). Toxins produced by cHABs (e.g., microcystins, anatoxins, saxitoxins) are deleterious to macro- and microfauna. Some heterotrophic bacteria can effectively degrade cyanotoxin molecules and use them as a nutrient source. Here, we explored the idea that the demise of cyanotoxins in the environment happens through microbial degradation. Our overall goal was to characterize the associations between cyanotoxin-degrading heterotrophic bacteria, cyanobacteria, cyanotoxins, and environmental parameters. We analyzed metagenomic and metatranscriptomic data from twenty warm monomictic lakes, all prone to cHABs, distributed across a precipitation gradient in the southcentral USA, sampled in April (bloom season) and August (non-bloom season) in 2021 and 2022. We assembled 915 metagenome assembled genomes (MAGs) from these data. Of these, 33 MAGs were taxonomically assigned as cyanobacteria, with the genera *Planktothrix*, *Pseudoanabaena*, *Nostoc*, and *Cyanobium* identified as the main cyanotoxin producers. We identified 30 MAGs of heterotrophic bacteria of the Actinomycetota, Bacteroidota, Bdellovibrionota, Chloroflexota, Patescibacteria, Planctomycetota, Pseudomonadota, and Verrucomicrobiota phyla that are positively correlated with higher cyanotoxin concentrations in the lakes, and therefore have potential to be toxin degraders. We identified relationships between the cyanotoxin producers and degraders metabolisms, regulation pathways, and transport proteins, specifically with regards to the nitrogen cycle. This work will provide insightful information to provide guidelines for the prevention and mitigation of cHABs in freshwater bodies.

Fungal Infections

FI-1F

Regulatory systems controlling *Candida albicans*-host interactions

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Transcription regulators, *i.e.* proteins that control gene expression by binding to DNA in a sequence-specific manner, are key elements of the *Candida albicans* gene network driving proliferation in the mammalian host. While genetic screens have identified transcription regulators that explicitly contribute to the fitness of this fungus in mice, the biological processes that such regulators control remain, for the most part, undefined. I will present our findings on two of such transcription regulators, Rtg1 and Rtg3, which we have established are key determinants of sphingolipid homeostasis in *C. albicans*. Quantitative analysis of the *C. albicans* lipidome revealed Rtg1/3-dependent alterations in all complex sphingolipids and their precursors, ceramides. Mutations in the regulators rendered the fungus susceptible to myriocin, a sphingolipid synthesis inhibitor. Rtg1/3 exerted control on the expression of several enzymes involved in the synthesis of sphingolipids' building blocks. More recently we have established that these regulators are activated upon engulfment

FI-2F

Protection vs Pathogenesis: Understanding the role of the antimicrobial peptide cathelicidin during fungal sepsis

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When antifungal treatment fails, patient outcome during invasive fungal infection is determined by the balance between productive infection control and effective limitation of damaging host responses. By studying how both host and fungal factors contribute to immune dysregulation and damage in the context of fungal infection, we aim to identify and test viable targets for host-directed therapy. Employing both mouse models and cell culture systems to investigate fungal interactions with the host, we tested the hypothesis that disease severity and host susceptibility to fungal

infection can be driven by both pathogen virulence and host response. These studies identified the antimicrobial peptide cathelicidin as an underlying factor by which the host response exacerbates the symptoms of fungal sepsis during intravenous infection. We demonstrate that cathelicidin loss is associated with dampened macrophage response, decreased fungal burden and inflammation in the heart, and ultimately, enhanced survival compared to wild-type mice. Ongoing studies seek to dissect the mechanistic basis for these cathelicidin-regulated effects and to extend our observations to a clinically relevant model of poly-trauma associated fungal infection.

FI-3F

Human skin as a reservoir for *Candida auris* and ESKAPE pathogens: transmissible microbes and antibiotic resistance in nursing homes

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Nursing home residents are at increased risk of colonization and infection due to antimicrobial-resistant bacteria and fungi. Nursing homes act as reservoirs, amplifiers, and disseminators of antimicrobial resistance within healthcare networks and across regions. Here, we sought to investigate the genomic epidemiology of the emerging, multidrug-resistant human fungal pathogen *Candida auris* in a ventilator-capable nursing home in Chicago, IL, USA. Coupling strain-resolved metagenomics with isolate sequencing, we report skin colonization and clonal spread of *C. auris* throughout the Chicago metropolitan region. Additionally, we report sharing of a large constellation of ESKAPE (*Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa)* and other high-priority pathogens (including *Escherichia coli, Providencia stuartii*, *Proteus mirabilis*, and Mo*rganella morganii)* within a nursing home. Integrating microbiome data with clinical microbiology results, we detected carbapenemase genes at multiple skin sites on residents identified as carriers of these genes months prior. To assess the generalizability of our findings, we analyzed publicly available shotgun metagenomic sequencing samples (stool, skin) collected from residents of seven other nursing homes with varying levels of medical acuity in three other states, elucidating additional evidence of previously unappreciated bacterial strain sharing within nursing homes. Taken together, our data suggest the skin is a reservoir for colonization by *C. auris* and ESKAPE pathogens, and their associated antimicrobial resistance genes.

FI-4T

Cathelicidin modulates the host response during fungal sepsis

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Invasive fungal infections are a significant cause of global morbidity and mortality, with ~2 million deaths annually. These infections primarily affect immunosuppressed, critically ill, and elderly individuals, and increasing antifungal resistance complicates treatment. *Candida albicans* (*Ca*), a common commensal fungus, can cause severe systemic infections when immune balance is disrupted. Therefore, understanding host factors that contribute to damage during fungal infections is essential for improving patient outcomes. This study explores the role of cathelicidin (CRAMP), an antimicrobial peptide produced by epithelial cells and leukocytes, in modulating the host response to fungal sepsis. CRAMP directly kills pathogens and influences host cell activity by acting as a chemoattractant and inducing inflammation. The immunomodulatory roles of cathelicidin during acute systemic *Ca* infection, have not been investigated. Our findings reveal that loss of murine-CRAMP protects against acute systemic infection, allowing CRAMP-deficient mice to survive lethal doses of *Ca* that kill wild-type mice in 24hrs. CRAMP-KO mice exhibited lower serum alanine aminotransferase and potassium levels, indicating reduced liver damage. Notably, the CRAMP-KO heart showed significantly lower fungal burdens, associated with reduced inflammatory cytokine production. This suggests that CRAMP deficiency improves survival during *Ca* sepsis by modulating inflammatory responses and reducing tissue damage. Current work involves histological analysis of PAS-stained cardiac tissue to quantify *Ca* and inflammatory infiltrates with a segmentation pipeline. These data underscore CRAMP's immunomodulatory role and highlight the potential of targeting CRAMP-mediated inflammatory pathways as a therapeutic intervention in *Ca* sepsis. Further research is needed to explore cardiac-specific effects and optimize treatment strategies.

VBP-1F

Characterizing bacterial behavior during human infection to guide new discoveries

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Bacterial behavior and physiology during human infection is difficult to study and largely unknown, as our vast knowledge of infection microbiology is primarily derived from studies using in vitro and animal models. A key challenge to assessing bacterial physiology during human infection is the difficulty in acquiring and assessing bacterial function in human-derived samples. Here, I will discuss the use of microbial metatranscriptomics from chronic human wound, lung, and oral infections to tackle this gap in knowledge. We have leveraged these data in two primary ways: to assess and improve the accuracy of pre-clinical infection models using a quantitative framework recently developed in our lab; and identifying and functionally characterizing genes of unknown function that are highly expressed in humans but not in most pre-clinical models. I will also discuss additional approaches we are using to quantify microbial biogeography and heterogeneity within human infections, with the goal of using these data to develop accurate pre-clinical models for antimicrobial discovery.

VBP-2F

Identification of Trim47 as a strain specific restriction factor for murine norovirus infection

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Human Norovirus is the leading cause of gastroenteritis worldwide. There are not any approved vaccines or therapeutics for treating norovirus infections, largely due to the intractability of human norovirus to in vitro culture conditions and lack of a small animal model. Murine norovirus (MNV) has emerged as the premier norovirus model system due to its similarity with human norovirus, the ability to propagate MNV in immortalized cell lines, its natural host are laboratory strains of mice. An outstanding question in the norovirus field is how norovirus genetic diversity impacts infection outcomes in vivo. We hypothesize that host restriction factors function to shape the ability of different norovirus strains to infect cells and cause disease. To systematically identify anti-norovirus genes, we conducted a genome-wide CRISPR activation screen with different MNV strains. We identified two proteins of the tripartite motif-containing (TRIM) family that have potent anti-norovirus activity: Trim7 and Trim47. TRIM proteins are E3-ubiquitin ligases and have been associated with host-viral interactions. However, very little is known about Trim7 and Trim47. While Trim7 displays broad anti-norovirus activity, Trim47 selectively inhibits MNV strains that cause persistent infection. Leveraging a viral evolution pipeline, we identify mutants of MNV that are resistant to Trim47. We determine that the variation in the non-structural protein 1 (NS1) accounts for differential sensitivity of MNV strains to Trim47 restriction. Further work suggests that NS1 is a substrate for Trim47. Our data provided new insight into antiviral genes and viral evolution that may impact viral tropism.

VBP-3T

DETECTION OF MACROPHAGE PHAGOCYTOSIS IN HUMAN NOROVIRUS INFECTION USING EX VIVO HUMAN INTESTINAL ENTEROIDS-IMMUNE CELL COCULTURE SYSTEM

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Human norovirus (HuNoV) causes acute gastroenteritis in immunocompetent hosts and chronic infection in immunocompromised individuals. Recent studies of innate immunity in the context of HuNoV infection utilized epithelium-only human intestinal enteroids, which lack immune cells. Here, we developed an *ex vivo* enteroid-immune cell coculture model consisting of human intestinal enteroids (HIEs) and human peripheral blood mononuclear cell-derived macrophages to better recapitulate the *in vivo* gut biology and explore the role of innate immune cells in HuNoV pathogenesis. Previous histopathological studies using intestinal biopsies derived from chronically infected immunocompromised individuals identified enterocytes and potentially enteroendocrine cells as the primary sites for HuNoV infection and replication. Additionally, HuNoV viral antigens were also found in macrophages, suggesting their role in eliciting an antiviral response. By performing HuNoV infection in HIEs cocultured with human peripheral blood mononuclear cell-derived macrophages, we showed that pro-inflammatory macrophages exhibit the highest potency in phagocytic activity for HuNoV-infected epithelial cells. These findings indicate a specific macrophage

phenotype readily recognizes HuNoV-infected cells and may play a protective role in controlling virus spread. The establishment of the macrophage/HIE co-culture model enables future studies to identify host cell molecules involved in immune-epithelial cell crosstalk and to develop preventative or treatment strategies to lessen the severe disease associated with HuNoV infection.

VBP-4T

Ebola infection activates lipid pathways for neutral lipid synthesis and lipid mediators in CD8$^+$ cells of *Mucaca mulatta*

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Ebola virus disease (EVD) can lead to severe symptoms with a high fatality rate (30-90%). In recent years there has been work on vaccines and antivirals to prevent or treat EVD. However, many of these vaccines or treatments are in various stages of development and are not yet widely available. Therefore study of the pathology of EVD is required to better understand the physiological effects of Ebola infection so that novel therapeutics can be developed. In this study we used cutting edge mass spectrometry technology to analyze the proteomics and lipidomics of isolated *Mucaca mulatta* CD8$^+$ cells following Ebola infection. When we performed an integrated multiomics analysis with lipid pathway analysis we found that there was an activation of triacylglycerol synthesis via the Kennedy pathway which likely leads to an increase in intracellular lipid droplets. Additionally, there appeared to be increased fatty acid remodeling within phosphatidylcholines and phosphatidylethanolamines where polyunsaturated fatty acids (PUFA) were significantly decreased and enzymes with specificity for PUFA were significantly upregulated. Proteomics also found an upregulation of enzymes involved in lipid mediator synthesis, signaling molecules that activate downstream immune responses which are synthesized from PUFA. There was also an upregulation of lipid droplet proteins that are known to possess antiviral activities. Taken together our integrated proteomics and lipidomics analysis suggests an activation of lipid synthesis and lipid modifying enzymes that primes CD8$^+$ cells to combat Ebola infection intracellularly via antiviral lipid droplets and more broadly by activation of immune responses via lipid mediators.

VBP-5F

Transcriptional heterogeneity in microbial gene regulation and pathogenesis

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While it has been recognized for some time that gene expression varies substantially within even isogenic populations of microbes, and that this has important implications for microbial biology and pathogenesis, it is only recently that technologies have emerged for profiling transcript abundance in single cells on a genome-wide scale. Using bacterial single cell RNA sequencing (scRNA-seq), we recently found that a major driver of transcriptional heterogeneity in proliferating cells is the replication of the bacterial chromosome. By resolving cell cycle expression patterns in unsynchronized *Escherichia coli* and *Staphylococcus aureus* populations based on single cell gene expression, we present evidence that while replication universally perturbs transcription, it does so in recurrent ways that depend on the regulatory architecture of individual genes. This includes simple gene copy number effects sensitive only to replication timing and transcript degradation rates, but also replication-associated activation of repressed genes and replication-associated repression of genes far from their promoter. At a global scale, modeling transcriptional heterogeneity enables quantitative analysis of other features such as replication patterns, transcription elongation speeds, and division timing. Hence, scRNA-seq represents a new tool that, when combined with effective analysis frameworks, can provide rich new insights into microbial gene regulation and physiology. In my lab, we are now starting to consider how these tools can also be leveraged to understand phenotypic heterogeneity in the context of bacterial pathogenesis.

UNDERGRADUATE POSTERS

UG General Microbiology:

UGP 1

Sequencing the genomes of environmental bacteriophage samples targeting *Pseudomonas Aeruginosa*

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As antibiotic regimens become less effective, bacteria-infecting viruses known as bacteriophages are gaining increased interest as an alternative treatment method. This project aims to isolate a bacteriophage (phage) that targets and eliminates a strain of the bacteria *Pseudomonas aeruginosa* known as PA14 and then sequence its genome. *P. aeruginosa* infections are one of the most common hospital-acquired infections and most commonly occur in people with compromised or otherwise weak immune systems, such as people with cystic fibrosis. Using an environmentally collected sample, we propagated and isolated the phage before performing DNA extraction and sent the genome to be sequenced using Illumina HiSeq. To process the genome, we used a variety of programs in combination with each other, such as Fastp, Bowtie2, SPAdes, and others, to clarify and highlight the genes we wished to examine. Beyond the interest in possible therapeutic application, we also aim to characterize this virus's genetic makeup, structure, and general behavior of this virus to understand how it behaves and responds to changes in selective pressure, which may prove useful for future phage studies and identifying shared characteristics across viral samples.

UGP 2

Revisiting Campylobacter plasmids: a pangenome analysis

Aurelio C. Del Carmen, Madison M. Schultz, Todd P. Primm, Anand B. Karki
Sam Houston State University, Huntsville, USA

Campylobacter species are important foodborne pathogens causing gastrointestinal diseases and are commonly found in retail meats. *Campylobacter* plasmids are well-known carriers of antimicrobial resistance genes and virulence genes and have been categorized by previous work into four main groups according to their genetic content. The number of available sequenced *Campylobacter* plasmids in the PLSDB (plasmid database by Schmartz et al., at Saarland University) and Genbank has increased from 133 to 362 since the previous categorization performed in 2018. The objective of this study is to revisit the pangenome analysis of available *Campylobacter* plasmids. All 362 *Campylobacter* plasmid sequences available in the PLSDB database (v.2023_11_03_v2) were annotated (Prokka, v.1.11), and pangenome analysis (Roary, v3.11.2) was carried out. Plasmids are categorized according to their genomic content. This study helps to understand the changes in the pangenome of *Campylobacter* plasmid over this short period. Likewise, it will help to determine the validity of the previous categorization and whether new categories should be created to harbor plasmid variations.

UGP 3

FlyingTortilla, ScarletRaider, BluerMoon, and EvenBluerMoon: Characterization of Novel Actinobacteriophages Isolated in Lubbock, Texas

Christian DeLuna[1,2], Carson Bellew[1,3], Francesca Makilan[1,4], Natalie Block[4,3], Whitney Dickens[4,3], Laurissa Miller[3], Allie Smith[3], Lisa Bono[4]
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An area of interest in science is bacteriophages (bacteria-infecting viruses) because of their potential usefulness when combating bacteria that have become resistant to traditional antibiotics. The HHMI Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program was implemented at Texas Tech through the TrUE Scholars Undergraduate Research Program. Soil was sampled from various locations around Lubbock, TX with the goal of identifying novel phages. From the raw soil sample, several isolation methods were used to identify novel phage compatible with bacterial hosts Gordina rubripertincta and Anthrobacter globiformis. We then went through the process of purification and amplification to ensure that we have a high concentration of pure virus for downstream applications. Our efforts culminated with the discovery of four novel phages, FlyingTortilla, ScarletRaider, BluerMoon, & EvenBluerMoon. Viral genomes of our novel phages were extracted and sequenced in collaboration with the University of Pittsburgh and SeqCoast Genomics. and subjected to genomic annotation. This data will be used to contribute to the knowledge of viral ecology to the broader scientific community

UGP 4

Evaluating the Impact of Washing Techniques on Microbial Reduction and Antibiotic Resistance in Texas Blueberries

Samuel Demaio, Ronald Wanja, Sin Man Mak, Megan Romeo
Dallas College, Dallas, USA

This study investigates the effectiveness of different washing techniques on microbial reduction and antibiotic resistance in blueberries, focusing on their implications for food safety and public health. Four washing methods were evaluated: no wash, water wash, a commercial fruit and vegetable wash ("Fit organic"), and a homemade baking soda and vinegar solution wash. The wash methods were assessed for their ability to reduce microbial colonies on blueberries purchased from a Dallas, TX supermarket. Microbial cultures were grown from swabs taken post-washing, with serial dilutions performed to facilitate colony counting. Our results show significant reduction in microbial colonies for all washing methods compared to unwashed controls, with the baking soda and vinegar solutions performing the best. Notably, one of the microbial types, identified as "Round Yellow", displayed resistance to streptomycin. This discovery highlights the potential for washing techniques not only to reduce microbial load, but also to influence antibiotic resistance profiles among surviving microbes. The introduction of the study contextualizes these findings within broader food safety research, emphasizing the necessity of optimizing washing methods to enhance microbial safety in food products. Future directions include further characterization of the microbial species found and refinement of washing solutions to maximize efficacy and minimize antibiotic resistance.

UGP 5

Elucidating the effect of Plasticizer Phthalates (DEHP) on Kaposi's Sarcoma associated Herpesvirus Infection

Spandan Mukherjee, Erica L. Sanchez
University of Texas at Dallas, Richardson, USA

Kaposi’s Sarcoma-associated Herpesvirus (KSHV) causes Kaposi’s Sarcoma (KS). KS has a high prevalence in Sub-Saharan Africa and AIDS-infected patients worldwide. Phthalates, like Di-(2-ethylhexyl) phthalate (DEHP), are additives widely used to soften plastics in many products including toys, shower curtains, and cosmetics. In particular, DEHP is implicated in many oncogenic and viral processes. My project focuses on the interaction between DEHP and KSHV. We hypothesize that DEHP will cause differential gene expression in KSHV-infected cells and affect virus production. All herpesviruses have both latent and lytic infection stages. Latency is defined by minimal viral gene expression and no virion production, while lytic replication expresses the complete viral genome and produces virions. I used a lytic inducible, latently KSHV-infected cell line to create virus particles with exposure to DEHP. When presented to uninfected cells, preliminary results show that DEHP exposure leads to changes in infection. Identifying possible DEHP and KSHV interactions by synthesizing a list of target genes affected by both KSHV and DEHP is key. I will measure whether DEHP treatment transcriptionally regulates these key genes through RT-qPCR, to establish a possible pathway of action. My expected results are increased KSHV replication measured by more abundant infectious virus and upregulated KSHV genes in the presence of DEHP. However, a measured decrease in virions or gene expression could indicate that DEHP inhibits viral replication. Identifying DEHP-affected host and viral mechanisms will be a critical step in determining the impact of widespread phthalate use on KSHV-infected cells and KS patients.

UGP 6

Virus-host interactions in warm monomictic lakes across southcentral USA: deciphering the potential ecological implications of viral infections

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Viruses, specifically phages, are the most abundant and diverse ‘life forms’ on Earth. Viral infections play a valuable role in nutrient cycles via host lysis that recycles nutrients or the encoding of auxiliary metabolic genes. Virus-host relationships also serve a critical role in evolution as vectors of horizontal gene transfer. To explore the relationships between viruses, their hosts, and their environment, we searched for viral sequences in metagenomic datasets of the host fraction (>0.22 µm) from 20 lakes located in the southcentral USA. The locations of these lakes spanned a strong precipitation gradient and had variable watershed land-use practices. The lakes were sampled during the months of April and August in 2021 and 2022. All the lakes are classified as eutrophic and are prone to harmful algal blooms. We calculated the ratio between lytic and lysogenic infections, which can impact nutrient recycling and microbial adaptation, respectively. These ratios were compared to the measured environmental factors to understand the

factors that drive viral infections, as well as further our understanding of the role of viruses in these freshwater ecosystems. We also identified the auxiliary metabolic genes encoded by these viruses to evaluate their potential contribution to the carbon, nitrogen, and phosphorus cycles. Finally, we estimated viral abundances and identified potential hosts to describe virus-host dynamics. This project will allow us to better understand the respective roles viruses and hosts have in their environment, and the outcomes of those interactions.

UGP 7

Using Transposon Sequencing (Tn-Seq) to identify essential genes for temperature-dependent biofilm formation in *Pseudomonas aeruginosa*

Vincent Mercado, Alex Luecke, Catherine Wakeman
Texas Tech University, Lubbock, USA

Pseudomonas aeruginosa can be isolated from a variety of locations such as soil or bodies of water and is known to cause infections in various hosts, such as plants and animals. These infections are complicated by this organism's ability to form biofilms, which can dramatically increase antibiotic tolerance. Previous research has shown that *P. aeruginosa* biofilms are structurally different in response to temperature shifts. By elucidating the mechanisms that drive these temperature-dependent biofilm adaptations as *P. aeruginosa* transitions from one niche to another, we can potentially identify novel drug targets.
To address this, we conducted a Transposon Sequencing (Tn-seq) analysis to identify mutants that are less fit in *P. aeruginosa* biofilms in response to temperature stress. In this study, each mutant has a single nonfunctional gene from a transposon insertion event. The mutants were then pooled, cultured at 23˚, 30˚, 37˚ and 40˚C for 48-hours, and then sequenced. In downstream analysis, mutants sequenced less were a result of lower abundance in the pooled culture after temperature stress and therefore less fit. From this, we have been able to characterize essential genes for growth in both environmentally and clinically relevant temperatures. For example, we identified a temperature-dependent preference for type 4 pili utilization at host temperatures and chaperone-ushered pili utilization at environmental temperatures, both of which have been described to facilitate surface attachment in the early stages of biofilm formation. We aim to continue studying these proteins to elucidate the mechanism for this temperature-dependent phenotype.

UGP 8

Investigation of Bacterial Content of a Commercial Skin Probiotic

Yaseen Eleyan, Katelyn Perez, Analia Tovar, Claire Edwards
St. Edward's University, Austin, USA

The cosmetic usage of probiotics is a growing phenomenon concerning the acquisition of a healthy skin microbiome to combat various conditions. The use of probiotics can improve an individual's natural skin barrier. However, the metrics by which their efficacy and quality are evaluated remain to be determined. Using the Eliderm Skin Probiotic, our research aimed to verify the bacterial contents of this product. The probiotic was cultured on Mannitol Salt agar [MSA], De Man–Rogosa–Sharpe agar [MRS], and Yeast Extract Peptone Dextrose agar [YPD] to determine what types and concentrations of microbes were present. YPD indicated the presence of total microbes, MRS quantified *Lactobacillus* spp., and MSA quantified Staphylococcus spp. or other halophiles. The results indicated that MRS media exhibited a higher average microbial count compared to MSA (252 ± 181 cfu/mL and 30 ± 35 cfu/mL, respectively) but a very similar average to YDP (231 ± 126 cfu/mL), suggesting a large presence of *Lactobacillus* spp. in the probiotic. This highlights the predominance of potentially beneficial Lactobacillus spp. in the probiotic formulation. The variation among the three experimental groups was considerably large, which may be due to the low bacterial concentrations. This could result in inconsistent application of probiotics during use. Ultimately, these results validate the importance of using multiple media types in order to assess microbial diversity and presence accurately. These findings reaffirm the need for further investigation of skin probiotics regarding the efficacy of their cosmetic usage, as every human possesses a unique microbiome.

UGP 9

Protocol Development for Identification of *Staphylococcus aureus* from Wastewater

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Wastewater can be a source of human-associated microbes which can tell us about the health of a community. This study aims to develop a protocol to isolate MRSA (methicillin-resistant *Staphylococcus aureus*) from wastewater

samples and to identify the types of MRSA found. We receive weekly wastewater samples from different treatment plants around the greater San Antonio region and we are using them to design our protocol which is in the early stages of development. We begin by preparing Baird Parker Broth and mixing it with our wastewater sample. The broth and wastewater mixture is incubated in a shaker overnight and then streaked onto Baird Parker agar, which is selective for Staphylococcus, and incubated overnight. Of the colonies that form, eight colonies are selected and patched onto a mannitol salt agar (MSA) plate. After overnight incubation, we identify all the colonies that grow and ferment (presumptive *S. aureus*) on the MSA plates. Our next step is to make glycerol stocks and prepare simple boil preps for PCR and eventual sequencing. We are currently optimizing our PCR methods to identify not only the type of Staphylococcus species present but also to determine the strain of MRSA present. So far we have isolated 62 fermenting colonies and in the coming weeks, we will send our samples for sequencing. Our protocol will be developed further over the remainder of the Spring semester.

UGP 10

High prevalence of *Campylobacter* and *Enterococcus* as co-contaminants in retail chicken liver products.

Madison M. Schultz, Aurelio C. Carmen, Marwa Hemri, Marbella Canaca, Marcos Montelongo, Layla R. Behrens, Alondra Lugo, Jesus Alvarez, Stanlee Brandt, Todd Primm, Anand Karki
Sam Houston State University, Huntsville, USA

Campylobacter and *Salmonella* remain the leading causes of gastrointestinal foodborne disease in the United States. Poultry is one of the most consumed meat products in the country, and consumption of contaminated retail poultry products is a major cause of clinical cases. *Enterococcus* species are found as normal microbial flora in human and poultry gut; however, their potential as opportunistic pathogens and carriers of antimicrobial resistance genes is a significant concern. The objective of this study is to determine the prevalence of *Campylobacter*, *Salmonella*, and *Enterococcus* in retail chicken liver products purchased from the Walker County (TX, USA) area, as well as further characterize these isolates using molecular approaches. A total of 106 retail chicken liver samples were collected from multiple grocery stores between June and October 2024. Foodborne pathogens were isolated using standard microbiological procedures and confirmed by polymerase chain reaction (PCR) with genus- or species-specific primers. The prevalence of *Campylobacter, Enterococcus*, and *Salmonella* was found to be 83%, 80%, and 29%, respectively. Among the tested retail liver samples, *C. jejuni* (49%) was more prevalent than *C. coli* (18%). *Campylobacter* and *Enterococcus* co-occurred in 68% of the retail liver samples, whereas only 27% of the samples had *Enterococcus* and *Salmonella* as co-contaminants. In conclusion, the high prevalence of *Campylobacter* and *Enterococcus* together as co-contaminants found in retail liver products is concerning. Ongoing studies focus on the antibiotic resistance of these strains and molecular characterization of selected isolates through whole genome sequencing.

UGP 11

Bioinformatic Analysis of Rhomboid Proteases in Pathogenic Protozoa

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Rhomboid proteases, a family of intramembrane serine proteases, are crucial in various biological processes across different organisms. Rhomboid proteases are highly conserved, indicating their essential role in biological functions. They are involved in critical processes such as cell signaling, host-pathogen interactions, and protein trafficking. Purpose: In this study, we investigated the conservation, structure, and function of rhomboid proteases in five pathogenic protozoans: Plasmodium, Acanthamoeba, *Trichomonas*, *Toxoplasma*, and *Leishmania*.

Methods: Phylogenetic trees were built using MAGAX, sequence homology studies performed using M-Coffee, and protein structure prediction models built using ExPASy to determine areas of conservation.

Results: Aligned sequences and structural comparisons reveal conserved motifs and functional domains, indicating evolutionary connections and functional similarities among the studied protozoans. *Acanthamoeba* shared traits with *Plasmodium* and *Toxoplasma*, suggesting similar molecular mechanisms in disease causation, while *Trichomonas* exhibited unique features that highlight its specific biological requirements. The conservation of rhomboid proteins in *Leishmania* suggests a role in the parasite's virulence and host-parasite interactions. Our study highlights the evolutionary significance and potential therapeutic targets of rhomboid proteases in pathogenic protozoa. Understanding their mechanisms can provide insights into developing targeted therapies for diseases caused by these parasites.

UG Pathogenic Microbiology Category:

UGP 12

Developing a Model System for Campylobacter Gut Infections

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Galleria mellonella, the Greater Wax moth, has been increasingly used as an animal infection model for infectious disease, with organisms such as *Staphylococcus*, *Clostridium*, and *Pseudomonas*. Worms can survive in conditions mimicking the human body, share innate immune system similarities, are small and inexpensive, and have short life cycles. Our goal was to further develop the larvae model for pathogen/microbiome interactions. *Campylobacter jejuni* is the most common foodborne pathogen in humans, usually transmitted in meats. Several research groups have published infections of worms with *Campylobacter*, however these involved the standard method of injecting the bacteria into the body cavity. In order to have a more relevant model, we are building an oral infection system, which also allows us to look at interactions between the *Campylobacter* with other foodborne or gut microbes. The larvae were always ordered from the same supplier (Carolina), always stored the same size glass dishes, with containers sterilized before use. We compared worm rearing with various food preparations and at multiple temperatures. The bruising, weight, viability, movement, and silk output was observed in these varying conditions in order to determine the optimal environmental conditions. It was found that 40 C is the thermal max with 41C being lethal. No weight gain or pupating was observed at 15C with food, but weight gain with delayed pupation was observed at 25C. We plan to use this model to explore polymicrobial interactions in the animal gut and to discover factors that stimulate or inhibit *Campylobacter* virulence in the gut.

UGP 13

Characterization of Novel Genes Critical for Iron Acquisition from Hemoglobin in *Bacillus anthracis* Sterne

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Bacillus anthracis is a spore-forming, gram-positive bacterium that is known to be the causative agent of anthrax. Due to its high mortality rate upon inhalation and threat as a biological weapon, understanding *B. anthracis* virulence mechanisms is of interest. To survive and proliferate within a host, *B. anthracis* must escape the host's immune response and obtain nutrients, such as iron. We used a previously created transposon library to screen for mutants that are unable to grow when the only source of iron is hemoglobin. In a screen of 2000 mutants, approximately 20 were unable to acquire iron from hemoglobin. Two of these mutants, 9F12 and 4E12, were sequenced and found to have independent mutations in the same gene that encodes for the dUTPase enzyme. This enzyme catalyzes the hydrolysis of dUTP to dUMP and is crucial for the integrity of DNA. There is no obvious connection between iron acquisition and dUTPase, however, we have verified its role through complementation. We are currently confirming the phenotype of the remaining transposon mutants pulled from our original screen. We have identified 4 additional mutants that are unable to grow when hemoglobin is the sole source and do not have a mutation in the *dUTPase* gene. In the future, we will determine which genes are disrupted in our remaining mutants and confirm the phenotypes through complementation and/or construction of independent mutants.

UGP 14

SCV Formation as a Mechanism for Nafcillin Resistance in S. Aureus

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading bacterial cause of death in 135 countries and has proven to be a challenging pathogen to treat due to its propensity for high levels of antibiotic resistance. As the development of new antibiotics has stalled, new approaches are needed to understand and counter the evolution of antibiotic resistance. Here, we uncovered a novel nafcillin (NAF) resistance pathway characterized by a small colony variant (SCV) phenotype in laboratory evolved nafcillin resistant MRSA. In literature, SCVs have been described as slow growing to aid in evading immune responses, thus they are often linked to persistent and relapsing MRSA infections. Consistent with previous studies, our nafcillin-resistant SCVs exhibited poor fitness and enhanced biofilm formation. To corroborate that SCV formation is related to nafcillin resistance, the SCVs were passaged in the absence of the drug to encourage reversion to the normal colony variant (NCV) phenotype. The NCVs lost their

nafcillin resistance, but still showed enhanced fitness. Additionally, the NCVs exhibited decreased biofilm production. Nafcillin targets peptidoglycan synthesis, which leads to collateral effects on bacterial membranes. On further inspection, nafcillin resistant SCVs exhibited increased membrane permeability compared to the wildtype, which on reversion, remained elevated in the NCVs. These findings suggest a link between persistent membrane alterations associated with SCV formation and nafcillin resistance. Future efforts will explore the genetic alterations in the resistant SCVs to map the evolutionary path taken to gain nafcillin resistance.

UGP 15

Developing Local Delivery of Phage to Treat Antibiotic-Resistant Staphylococcus aureus Biofilm Osteomyelitis

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Staphylococcus aureus is the most common cause of osteomyelitis (OM, bone infections) associated with implanted orthopedic devices due to biofilm formation of the surfaces. Biofilms are inherently antibiotic resistant and coupled with the general rise of antimicrobial resistance (AMR), antibiotics are becoming ineffective for OM treatment. A possible alternative OM treatment is bacteriophage (phage), which are viruses that selectively infect and lyse bacterial cells and do not target human cells. We are investigating local delivery of phage encapsulated into biodegradable microspheres for OM treatment to address the poor blood flow to OM infectious. We have shown that microspheres containing the anti-staphylococcus phage K are active against the *S. aureus* clinical isolate UAMS-1. Unfortunately, our current rate of phage K incorporation into microspheres (0.01%) is less than optimal for treatment. We hypothesized that increasing the concentration and purity of phage to be encapsulated would increase the incorporation efficiency. By altering steps in a standard phage purification protocol including optimal growth medium, chloroform addition, and CsCl density gradient ultracentrifugation, we obtained high purity phage K stocks of up to 2 x 10^12 PFU/ml, which were two orders of magnitude greater than those from plate lysates. However, to date the use of increased phage concentrations did not increase our incorporation efficiency. Our future plans include identifying the step(s) responsible for the loss of phage activity or poor incorporation. We will track fluorescently-labeled phage at each step and test phage sensitivity to the chemicals used in microsphere synthesis.

UGP 16

Determining DGAT2's role in maintaining lytic KSHV infection

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University of Texas at Dallas, Richardson, USA

Kaposi's Sarcoma-associated Herpesvirus (KSHV) is an oncogenic virus that causes Kaposi's Sarcoma (KS). Although endemic to Sub-Saharan Africa, it is the most prevalent cancer among AIDS patients globally. It has been widely reported that lipid metabolism is required to maintain latent and lytic KSHV infection. The mechanisms of lipid modulation during KSHV infection have yet to be defined; however, previous studies from our lab and others have explored changes in lipid storage. This implies that KSHV-infected cells prioritize the production of triacylglycerols (TAGs) during the lytic phase of infection, which suggests that TAG synthesis is connected to maintaining KSHV lytic infection. Diacylglycerol O-acyltransferase 2 (DGAT2) is an imperative protein for effective TAG synthesis, as it is one of two final catalysts in the pathway responsible for the conversion of diacylglycerol (DAG) to TAG. Despite DGAT2's role in the TAG synthesis pathway, its significance during lytic KSHV infection is unknown. Preliminary relative mRNA expression data using a KSHV reporter cell line, iSLK.BAC16, were obtained through the usage of quantitative polymerase chain reactions (qPCRs). Statistical analyses of this data suggest consistent upregulation of DGAT2 during lytic KSHV infection. Therefore, we hypothesize that DGAT2 upregulation is required for maximal lytic replication. DGAT2's significance in maintaining lytic KSHV infection will be investigated through the use of qPCRs to measure known lytic KSHV-associated genes' expression once the knockdown or inhibition of DGAT2 occurs. Overall, DGAT2 has the potential to serve as a promising host target of KSHV infection and, by extension, antiviral drug design.

UGP 17

Modulating the Human Gut Microbiome with Gochujang, a Fermented Soybean Product

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The gastrointestinal tract hosts a diverse ecosystem of microorganisms, collectively known as the gut microbiome, which plays key roles in nutrient absorption, immune system development, pathogen defense, and communication with other organs. The microbiota and its host share a mutually beneficial relationship, with the microbiome supporting essential bodily functions that contribute to overall health. Everyone's microbiome is unique and influenced by factors such as diet, gender, and medication use.
In this study, we examine the effects of Gochujang, a traditional fermented soybean product (FSP), on the gut microbiota. While previous studies have shown that FSPs can positively impact both gut and overall health, limited research has explored specific effects of Gochujang on the human gut microbiome. To address this gap, we conducted a human subject study with 32 participants who provided fecal samples before, during, and after Gochujang consumption. Using 16S rRNA sequencing targeting the V3/V4 region, we extracted genomic DNA (gDNA) to assess changes in microbial diversity and composition over time. Our findings revealed noticeable changes in alpha and beta diversity, with shifts observed in key phyla such as Firmicutes, Bacteroidota, Actinobacteria, and Proteobacteria. These results suggest that, as a FSP, Gochujang can positively influence the gut microbiome by altering microbial communities.

UGP 18

Elucidating the effect of Plasticizer Phthalates (DEHP) on Kaposi's Sarcoma associated Herpesvirus Infection

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Kaposi's Sarcoma-associated Herpesvirus (KSHV) causes Kaposi's Sarcoma (KS). KS has a high prevalence in Sub-Saharan Africa and AIDS-infected patients worldwide. Phthalates, like Di-(2-ethylhexyl) phthalate (DEHP), are additives widely used to soften plastics in many products including toys, shower curtains, and cosmetics. In particular, DEHP is implicated in many oncogenic and viral processes. My project focuses on the interaction between DEHP and KSHV. We hypothesize that DEHP will cause differential gene expression in KSHV-infected cells and affect virus production. All herpesviruses have both latent and lytic infection stages. Latency is defined by minimal viral gene expression and no virion production, while lytic replication expresses the complete viral genome and produces virions. I used a lytic inducible, latently KSHV-infected cell line to create virus particles with exposure to DEHP. When presented to uninfected cells, preliminary results show that DEHP exposure leads to changes in infection. Identifying possible DEHP and KSHV interactions by synthesizing a list of target genes affected by both KSHV and DEHP is key. I will measure whether DEHP treatment transcriptionally regulates these key genes through RT-qPCR, to establish a possible pathway of action. My expected results are increased KSHV replication measured by more abundant infectious virus and upregulated KSHV genes in the presence of DEHP. However, a measured decrease in virions or gene expression could indicate that DEHP inhibits viral replication. Identifying DEHP-affected host and viral mechanisms will be a critical step in determining the impact of widespread phthalate use on KSHV-infected cells and KS patients.

UGP 19

Understanding Polymicrobial Competition in Chronic Wounds Using Artificial Clots in Mice

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Many chronic infections are determined to be made up of more than one microbial species. When taking this into account, a simple broad-spectrum antibiotic is usually not sufficient to get rid of these pathogens; especially when a biofilm is present at the infection site. In polymicrobial infections, to truly understand the role of each pathogen and establish a better antibiotic regiment for treating these chronic infections, testing of bacterial communities in clinical models is key. For our research, we work with four species commonly isolated from chronic infections (*Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter* baumannii*, and Enterococcus faecalis*). When applying bacteria to our mouse models, having a transferrable clot in our wound media was key to successful replication of a clinical infection. Preliminary mouse infection data revealed inter-species competition between microorganisms, however, these experiments were performed only with communities containing *S. aureus* as it is the only species among our strains tested that contains a coagulase enzyme. To test communities without S. *aureus*, we devised the solution of using Instagel cold set gelatin for the creation of artificial clots used for the application of bacteria into mousewound beds. Once artificial blood clotting was achieved, we then began looking at the growth dynamics in a polymicrobial infection consisting of all four species with and without Instagelin the mice wound beds. We are now looking at the growth dynamics and competition between all four species in our chronic wound model with the goal of understanding polymicrobial growth dynamics in host infections.

UGP 20

Microbial Interactions and Environmental Conditions Influence Antibiotic Susceptibility

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Dynamic interactions between pathogens, antibiotics, and environmental conditions play a critical role in the effective treatment of bacterial infections. This study investigates how variations in microbial communities and growth medium influence the antibiotic susceptibilities of Enterococcus faecalis and Pseudomonas aeruginosa. In this research, we found that E. faecalis exhibited decreased susceptibility to cephalexin when co-cultured with Acinetobacter baumannii, with significantly elevated CFU/mL compared to the monomicrobial condition. Further experimentation demonstrated that co-culture with the supernatant obtained from A. baumannii after cephalexin exposure or in a transwell plate also increased E. faecalis survival, indicating that factors secreted by A. baumannii in the presence of the antibiotic contribute to recalcitrance. Additionally, we observed that P. aeruginosa required more than double the concentration of kanamycin to inhibit growth in minimal medium containing malonate (commonly found in the bloodstream of trauma patients) as compared to minimal media containing glycerol (found in lab media). This finding shows that nutrient availability can influence antibiotic efficacy. These results underscore the importance of considering environmental factors, such as nutrient conditions and microbial interactions when conducting clinical antimicrobial susceptibility testing (AST). Incorporating these factors can help predict recalcitrance more accurately and improve the selection of effective treatment regimens for persistent infections in a clinical setting. Accounting for these variables in AST will enable more precise and targeted clinical therapies, leading to better patient outcomes and reduced treatment failures in the hospital setting.

UGP 21

Analysis of Pneumolysin-Induced Damage and Repair in Bronchial Epithelial Cells in Vitro

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Pneumolysin (Ply), a crucial virulence factor produced by *Streptococcus pneumoniae*, plays a key role in pathogenesis of this opportunistic organism. Our recent *in vitro* studies, using human bronchial epithelial cells (Calu-3), indicate dynamic alterations in Calu-3 monolayers' paracellular permeability after exposure to different concentrations of PLY. In this study, we sought to investigate the hypothesis that exposure of Calu-3 cells to a sub-lytic concentration of Ply (5 µg/mL) triggers repair mechanisms to re-establish tight junction structure and function, however, the following accumulation of endogenous inflammatory cytokines pushes the cells towards an irreversible injury. To address our hypothesis, we measured transepithelial electrical resistance (TEER) in Calu-3 monolayers cultured in cell culture inserts. We found a drastic decrease in monolayer integrity within the first hour of Ply exposure, followed by a steady recovery between 1-5 hours, suggesting activation of cellular repair processes. However, TEER values decreased again after 10 hours of exposure to Ply, in an apparently secondary insult response. We now utilize immunofluorescence imaging to visualize these changes in relation to the tight junction protein Occludin. To evaluate the role of inflammatory cytokines in tight junctions injury, we will use ELISA to quantitate target inflammatory cytokines and chemokines produced by Calu-3 cells during PLY exposure. The study aims to deepen our understanding of cellular responses to Ply-induced damage, with implications for understanding pneumococcal infection pathology and potential therapeutic strategies.

UGP 22

Exploring the Role of Nucleotide Synthesis During KSHV Lytic Replication

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Kaposi's Sarcoma (KS) is a currently incurable endothelial cell-based cancer driven by Kaposi's Sarcoma Herpesvirus (KSHV), a large DNA virus. KSHV is endemic to Sub-Saharan Africa and the most common cause of tumors among immunocompromised HIV/AIDS patients worldwide. While KSHV remains predominantly latent within endothelial cells, we expect that its shift to lytic replication demands significantly more vital biosynthetic components from the host cell, including carbohydrates, lipids, amino acids, and nucleotides. Properly manufactured pyrimidines and purines are necessary for complete nucleotide synthesis, which are essential during viral genome replication. However, the exact role and downstream effects of nitrogenous base scarcity remains unclear during KSHV infection. We hypothesize that nucleotide synthesis is required during KSHV lytic infection and that loss of this pathway will

result in reduced infectious virion production. To induce this change, we administered a known thymidine and purine synthesis inhibitor, methotrexate (MTX), to our iSLK cell line (doxycycline-inducible reporter cell line that stably maintains the KSHV genome). If nucleotide synthesis is essential during lytic replication, we anticipate reduced viral gene expression of vGPCR and ORF59 (both early lytic genes) and K8.1 (late lytic gene). Currently, our data suggest that MTX may terminate both host cell proliferation pathways and early lytic transition in virus-induced iSLK cells. With MTX already clinically relevant and established on the market as an anti-cancer and DMARD drug, its prospects for treating KSHV infections are promising.

UGP 23

Functional Study of Profilin Isoforms Using a Vertebrate Model

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During early development, the Wnt family of evolutionarily conserved signaling molecules play important roles including cell fate determination, cell proliferation, cell motility, and establishment of the primary axis. Importantly deregulation of Wnt signaling has been implicated in numerous human pathologies including tumor formation and birth defects. In the non-canonical Wnt pathway, the protein Daam1 interacts with Dishevelled, which is necessary for gastrulation and neural tube closure. In a screen for effector proteins of Daam1, the Profilin protein was identified, which plays a role in actin polymerization and regulation of cell movement, shape, and division during development. While lower eukaryotes, such as fungi, yeast, and protozoa, typically possess a single profilin gene, multicellular organisms often express several profilin isoforms, demonstrating adaptability and functional diversity across species. In vertebrates, four main profilin genes have been identified: *PFN1*, *PFN2*, *PFN3*, and *PFN4*. *PFN2* undergoes alternative splicing, producing *PFN2a* and *PFN2b* isoforms. While *PFN* isoforms have been studied in mammals, birds, and amphibians, much about their role in fish remains unknown. With its unique advantages as a model organism, zebrafish *Danio rerio* offers an opportunity to explore the roles of *PFN* isoforms during vertebrate embryogenesis. Our RT-PCR analysis suggests that *pfn1* and *pfn2a/b* isoforms have distinct expression patterns within the developmental stages of zebrafish, suggesting non-overlapping distinct functional roles. Currently, we are functionally characterizing the role of zebrafish profilin isoforms during their early embryonic development using gain-of-function by mRNA overexpression, loss-of-function by translation blocker oligonucleotides, and generating knockouts using CRISPR-Cas9 technique.

GRADUATE STUDENT POSTERS

GS Envornmental Microbiology:

GSP 1

Surveillance for reticuloendotheliosis virus and lymphoproliferative disease virus in wild turkeys

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Reticuloendotheliosis virus (REV) and lymphoproliferative disease virus (LPDV) are oncogenic and immunosuppressive avian retroviruses associated with neoplastic disease in Galliformes. Birds infected with REV have increased coinfection susceptibility and the potential to act as reservoirs for viral transmission of at-risk flocks, such as the Attwater's prairie chicken. Our objectives were to continue surveillance nationally and determine changes in the prevalence of REV and LPDV in Texas counties. Blood samples from 479 Rio Grande Wild Turkeys (*Meleagris gallopavo intermedia*) trapped in 18 Texas and two Nebraska counties were screened for proviral REV or LPDV DNA. The REV 3’ LTR or the LPDV p31/CA gene was targeted by polymerase chain reaction. Positive samples were sequenced, and nucleotide similarity was confirmed by BLASTn queries. Approximately 1% of individuals were infected with REV (6/479), and 24% were infected with LPDV (116/479), including three coinfections. The results from the screening showed a similar prevalence of REV compared to previous surveys conducted in 2016-17 and 2018–2020. However, the prevalence of LPDV in affected counties increased by 25-60%. While we previously detected most LPDV infections in females, we detected a similar proportion of infections in males and females in the current survey. Further, we previously detected a similar proportion of LPDV infections in juveniles and adults;

however, in the current study, most infections were detected in adults. Additional studies are needed to assess the impact of LPDV on Wild Turkey reproduction and overall health.

GSP 2

Development of a LAMP assay to detect pBI143 - an abundant plasmid specific for human waste

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Contamination of waterways with human waste poses a significant public health threat; however, traditional methods for assessing microbiological water quality rely on culturing fecal indicator bacteria (FIB). This approach takes 24 – 48 hours and does not indicate the source of the waste. Molecular microbial source tracking methods have been developed, but these require expensive equipment and highly trained personnel. The plasmid pBI143 is a reliable indicator of human fecal contamination and loop-mediated isothermal amplification (LAMP) is a simple, sensitive method of detecting specific DNA fragments. Here we report a loop-mediated isothermal amplification (LAMP) protocol for detecting pBI143. Primers were designed to amplify a conserved region of this plasmid and used in a colorimetric assay. This assay showed a clear color, that was visible with the naked eye, with DNA purified from municipal sewage samples. The entire protocol took 20 minutes and parallel no-template controls did not show a color change. This suggests a simple, rapid method for identifying human fecal contamination. Since the only equipment requirement is a heating block, this LAMP protocol could particularly be advantageous for low-resource settings and could be adapted to point-of-sampling monitoring.

GSP 3

DFW Soil Bacteria Shed Light on Understudied Genomes

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In efforts to combat the rise of antibiotic resistance in bacteria, the isolation and molecular understanding of novel but understudied antibiotic-producing bacteria is paramount. To that end, 15 bacterial isolates have been isolated from the Dallas-Ft. Worth (DFW) metroplex area. Of these, 3 isolates of particular interest, from the species *Rossellomorea marisflavi, Streptomyces virginiae*, and *Streptomyces hydrogenans,* underwent additional bioinformatic analysis to help further the understanding of these otherwise understudied bacteria. *R. marisflavi* was found to have incredible conservation of gene clusters between strains, although having notable genomic islands and point mutations between strains. Additionally, *S. virginiae* and *S. hydrogenans* were difficult to compare bioinformatically and difficult to place taxonomically. The placement of *S. hydrogenans* also uncovered a gross misplacement of strains into the *S. venezuelae* taxon. Ultimately, this work shows a need to refine criteria for speciation within the *Streptomyces* genus and demonstrates that pairwise average nucleotide identity comparisons with currently applied thresholds may not always be indicative of speciation.

GSP 4

Application of Digital PCR and High-Throughput Sequencing of 16S rRNA to estimate the contribution of Human Waste to Tributaries of Galveston Bay

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Improperly maintained on-site wastewater treatment systems (OWTS), domesticated/wild animals, and agricultural/urban run-off can contaminate waterways. Fecal contamination is a major environmental and public health concern in economically disadvantaged communities in the Houston-Galveston area; however, few studies have attempted to track the source of fecal contamination in this metroplex. Here, we implemented digital PCR (dPCR), to quantify crAssphages, and nextgen sequencing of 16S rRNA amplicons, to track the source of contamination In Western Galveston Bay. Water samples were collected at 33 stations routinely sampled by the Texas Commission on Environmental Quality (TCEQ). Water samples were concentrated via membrane filtration, nucleic acids were extracted, purified with a commercial kit, and copies of crAssphages and Enterococci were assessed with a multiplexed dPCR. Copies of 16S rRNA genes were assessed with quantitative PCR, and amplicons, generated with universal primers, were sequenced on an Illumina system. Copies of crAssphages were three orders of magnitude higher in the OWTS samples than animal or water samples. High copies of crAssphages were observed in bayous that have been classified as impaired by TCEQ. Nonmetric multidimensional scaling (NMDS) ordination showed

coherent clusters that differentiated water, OWTS and animal samples. Clustering by NMDS appeared driven by environmental conditions (temperature, pH and salinity) and by the abundance of bacteria as assessed by qPCR. Copies of crAssphages and Enterococci did not significantly fit to the NMDS ordination plot. These results suggest that dPCR can detect contamination with human waste, but does not strongly influence the microbial community in the watersheds.

GSP 5

Rhizosphere Soil Fungal Community Responses to Nitrogen Addition Reflects Both Plant Genotypic and Heritable Trait Variations

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Plants form tight associations with soil microbial communities and these interactions regulate coastal ecosystem processes. The dynamics of these interactions, however, are increasingly altered by anthropogenic pressures such as nitrogen loading. While it is known that different plant species can shape the rhizosphere soil (soil around roots) fungi, the influence of intraspecific plant variations on fungal communities under environmental stress over time remains poorly understood. Leveraging a 100-year-old marsh seed bank, we investigate how genotypic and associated trait variations in *Schoenoplectus americanus,* a dominant plant in the Gulf Coast saltmarshes, shape rhizosphere fungal communities under elevated nitrogen (N) over time. Ancestral (plants grown from 100-year-old seeds) and modern descendant plant genotypes were subjected to high and low N levels in a common-garden experiment. Fungal communities were then characterized using amplicon-based sequencing. Overall, fungal diversity was higher in the presence of plants regardless of nitrogen conditions whereas fungal guild diversity was unaffected by plants or nitrogen. Fungal communities also differed according to plant genotype and age cohort depending on N levels, as modern genotypes exhibited a stronger influence on fungal diversity compared to ancestral genotypes. We further observed genotype x environment interactions. Taken together, these results suggest complex dynamics between plant genotypic traits and nitrogen addition shapes microbial communities, providing valuable insights that can aid in the conservation and management of coastal marsh ecosystems in the face of ongoing changes.

GSP 6

Soil organic matter and microbial extracellular enzyme activities vary across soil infiltration berm installations

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Soil microorganisms are the primary drivers of nutrient cycling within soils via nutrient mineralization of carbon and nitrogen. This function is largely driven by water and nutrient availability. In response to increased drought and water demand, the Edwards Aquifer Authority (EAA) in San Antonio, Texas is investigating if infiltration berm installation concomitantly increases both groundwater recharge and soil health. This project's efforts aim to assess soil microbial community composition (bacteria/archaea and fungi) and microbial extracellular enzymatic degradation potential of carbon and nitrogen under varying moisture conditions in soils across bermed and unmanaged plots. High throughput fluorometric assays revealed significant differences in hydrolytic enzymatic activity between baseline (control with no berm) and position within berm (β-glucosidase: $p = 0.0017$, β-xylosidase: $p = 0.00028$, Leucine aminopeptidase: $p = 0.025$, N-acetylglucosiminidase: $p = 0.025$, Cellobiohydrolase: $p = 0.00088$). The baseline site had higher soil organic matter than the bermed areas ($p = 0.0399$) but was comparable to the swale position within the berm. These data coupled with diversity indices and nutrient pools will reveal responses in soil health and microbial community dynamics associated with infiltration berm installation. Microbial community structure will be assessed using targeted analysis of 16S (bacteria/archaea) and ITS2 (fungi) genes for amplicon-based Illumina MiSeq sequencing. This work will elucidate the impact of berms on biological soil health, and likely the quantity and quality of groundwater recharge at the Edwards Aquifer Authority.

GSP 7

Soil health indicators of urban green spaces: addressing microbial aspects of soil fertility

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Soils provide essential ecosystem services with soil organic carbon (SOC) playing a critical role in maintaining soil health in the face of environmental change. SOC dynamics enhance environmental resilience by promoting microbial

activity and driving carbon sequestration processes. This project aims to deploy the Foldscope, an affordable and widely accessible paper microscope, across locations in Texas to expand microbial data collection and identify relationships between microbial properties (e.g., soil fungal biomass) and dynamic soil properties (DSPs). In collaboration with the Natural Resources Conservation Service (NRCS), this research utilizes the NRCS Soil Survey and Web Soil Survey to focus on DSPs, assessing how soil health indicators respond to anthropogenic disturbances like agricultural conversion and rangeland management. Preliminary soil data collected from urban green spaces (parks, walking trails, natural areas) in San Antonio were processed to assess gravimetric water content (GWC) and soil organic matter (SOM). A one-way analysis of variance (ANOVA) indicated that GWC did not vary among green space locations ($F_{6,32} = 2.03$, $p = 0.09$), but SOM varied among locations ($F_{6,32} = 7.57$, $p < 0.001$). Soil OM was significantly greater in most locations relative to one natural area (Sinkin Natural Area in northern San Antonio). Further data collection is underway, including fungal community composition via ITS2 targeted gene sequencing (Illumina MiSeq) and fungal biomass counts using microscopy and quantitative-PCR assays. These findings will be essential for understanding how microbial communities, particularly fungi, differ across urban soils in south-central Texas.

GSP 8

Viral Activity in Sediments from the South Atlantic Gyre

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Viruses are the most abundant biological entities in the ocean. They have been detected hundreds of meters below the seafloor and likely exist deeper. Viral infections are responsible for shaping microbial populations via the lysis of their hosts, which impacts nutrient recycling releasing dissolved organic carbon and nutrients back into the environment. Viral abundances in sediments range from 10^7 to 10^{10} per gram of sediment (cm^{-3}). The South Atlantic Transect (SAT) project collected sediments during four IODP expeditions in the western part of the Mid-Atlantic Ridge along ~30˚S latitude, providing a range of sediment types, geochemical gradients, and ages. This study compares the viral abundances and activity with physical and geochemical parameters to understand the importance of viral infections in deep-sea sediments. We calculated viral and cell abundances using epifluorescence microscopy in cores up to 122.6 m below the seafloor to estimate the virus-to-prokaryotes ratio (VPR) and evaluate the extent of viral infections. Additionally, we performed viral production experiments and induced prophages using mitomycin C for hole U1560, to determine the magnitude of viral infections in the microbial populations present. Viral abundances ranged from $1.58x10^4$ and $8.79x10^4$ virus-like particles (VLPs) cm^{-3}. VPRs of 28.47, 9.21, and 4.73 were observed in holes U1558, U1583, and U1560, respectively. Viral activity by lysis was more abundant at shallower sediments and decreased with depth while prophages were more prevalent at deeper sediments. Our results contribute to the understanding of the roles of viruses in shaping microbial populations and their relationship within deep-sea sediment.

GSP 9

Using NGS RNA-Seq to determine viral communities in arthropod populations.

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Arthropod-borne viruses (arboviruses) represent a significant threat to public health, necessitating the prompt detection of these pathogens within arthropod populations to enable rapid response measures. Polymerase chain reaction (PCR)-based techniques offer high specificity and sensitivity for the identification of target viruses. However, these methods are limited by their reliance on prior knowledge of target genomes, thus constraining the identification of novel viruses. Given that most arboviruses possess RNA genomes, researchers can employ next-generation RNA sequencing (RNAseq) for detection and characterization of mosquito virome. Defining the metavirome of a population enables the characterization of novel viruses and the assessment of virus distribution and interactions. Recent studies indicate that insect-specific viruses (ISVs) can inhibit the replication of arboviruses in culture and reduce their transmission in vivo. This interaction suggests a negative correlation between the abundance of ISVs and arboviruses in natural populations. In this study, we focus on three mosquito species: two native to Connecticut, *Culex pipiens* and *Culiseta melanura*, the primary vectors of West Nile Virus (WNV) and Eastern Equine Encephalitis Virus (EEEV), respectively, and a recently invasive species, *Aedes albopictus*. Mosquitoes were collected from multiple sites, sorted by species, and pooled for RNAseq analysis. Shannon diversity indices were employed to estimate richness and evenness within single population pools, while Bray-Curtis dissimilarity was used to compare species across all sites.

Our findings indicate that we successfully detected arboviruses within mosquito populations and established a negative correlation between arboviral abundance and the presence of ISVs.

GSP 10

Screening of Flies as Vectors and Sentinels of Antimicrobial Resistant Human-Foodborne Pathogens in Texas.

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This research aimed to analyze the spatial distribution of antimicrobial resistance (AMR) in distinct species of foodborne pathogens: *Salmonella* spp, *Listeria monocytogenes*, and *Campylobacter* spp. isolated from flies across the surrounding ecosystems. About 230 fly samples were collected from five locations in each of six counties in Texas during the first year. Three traps were positioned at distances of 100 m within a single sampling site. All the fly species captured in one trap were combined to create a single homogenized sample. Most fly species trapped were *Cochliomyia macellaria, Chrysomya rufifacies, Phormia regina, Lucilia sericata, and Musca domestica*. *Salmonella* spp. was detected in 89 samples, while *Listeria monocytogenes* isolated from 6 samples. To verify the presence of desired organisms in the samples, the 3M molecular detection system (MDS) was implemented following their detection from selective plates and selective enrichment. Antibiotic susceptibility testing was phenotypically assessed via Sensititre AST system. Pathogen isolates were serotyped by the U.S. Department of Agriculture's National Veterinary Services Laboratory concurrently with AST profiling. *Salmonella* was identified as *Salmonella infantis, Salmonella enteritidis, and Salmonella Montevideo* after serotyping, which are frequently present in broiler meat production and poultry, posing a significant risk to human health by inducing various gastrointestinal disorders. *Salmonella* isolates demonstrated resistance to chloramphenicol, nalidixic acid, streptomycin, and tetracycline, with a consistent resistance pattern across different counties. Flies appear to transmit antimicrobial-resistant foodborne -human pathogens into any surrounding ecosystems, hence surveillance of these insects may be beneficial for analyzing AMR gene transmission.

GSP 11

Effects of Bradyrhizobium Biofertilizer Application on Greenhouse Gas Emissions in South Texas

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Agriculture contributes approximately 10% of total greenhouse gas (GHG) emissions in the U.S. Included are carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), which are significantly contributing to climate change. The use of synthetic fertilizers is a major source of these emissions during both production and application, particularly N_2O, which is approximately 300 times more potent than CO_2 in terms of global warming. Biofertilizers, such as a drought-tolerant *Bradyrhizobium* strain (TXVA), have gained popularity as a sustainable approach to mitigating climate change. The soil bacterium forms a symbiotic relationship with the roots of leguminous plants such as soybeans (*Glycine max*), enabling them to fix atmospheric nitrogen (N_2) into ammonia (NH_3), which is available for plant use and reduce the need for synthetic fertilizers. In this study, 20-acre, non-irrigated soybean fields in South Texas were divided into plots with the TXVA biofertilizer and a no-treatment control. A Gasmet Analyzer with a Li-Cor Smart Chamber was used to quantify GHG fluxesthroughout the growing season to monitor CO_2, N_2O, and CH_4 emissions. Our objective was to evaluate and determine the impact of TXVA biofertilizer application compared to the nontreated control on GHG emissions. The findings contribute to our further understanding of the potential role of biofertilizers in mitigating agricultural GHG emissions.

GSP 12

Diversity of soil mycobiome in the gulf coast prairie dunes of barrier islands in South Texas

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Fungi play an important role in the soil mycobiome as key decomposers and in mutualistic plant-fungi interactions. Mycorrhizal fungi, including ectomycorrhizal (EcM) and arbuscular mycorrhizal fungi (AMF), are essential indicators of plant biodiversity and ecosystem resilience. Barrier islands face increasing environmental stresses, particularly under climate change, yet the diversity of mycorrhizal fungi in coastal prairie dunes in these islands remains underexplored. This study investigates the diversity of EcM and AMF in soils from four barrier islands along the South Texas Gulf Coast, focusing on gradients of salinity and vegetation types. We collected 5–20 soil cores from each

island and profiled the mycobiomes using high-throughput sequencing of the internal transcribed spacer (ITS) region, employing AMF and EcM-specific primers for a comprehensive survey. Soil properties, including total nitrogen content and geographic distance, were measured to assess their influence on mycorrhizal diversity and distribution patterns. Preliminary analyses reveal significant variability in soil properties and mycorrhizal diversity across the islands, with potential evidence of isolation by distance, indicating that both abiotic (soil) and biotic factors shape AMF and EcM communities. For example, EcM communities are significantly influenced by total soil nitrogen percent. These findings highlight the importance of understanding mycobiome dynamics in response to environmental changes to predict biodiversity loss accurately. Insights gained can inform coastal management and conservation strategies and contribute to nature-based solutions for rehabilitating barrier island habitats, such as promoting mycorrhizae-assisted belowground growth of coastal plants.

GS General Microbiology:

GSP 13

Breaking Up The Break Down: How An Open Reading Frame Inhibits Lysis In Bacteriophage N4

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Bacteriophage (phage), viruses that infect and kill bacteria, are reemerging as an important player in the fight against bacterial antimicrobial-resistant (AMR) infections. Successful AMR treatment with phage faces several challenges such as reproducible high-yield production. To overcome this block, we aim to repurpose natural strategies phages use to increase progeny yield. One phenomenon that allows phages to maximize host cell resources for progeny production is known as lysis inhibition (LIN). LIN is negative regulation of the phage lysis pathway in response to environmental conditions that increases the time available for progeny production. Phage N4 exhibits the LIN phenotype. N4 phage infections yield approximately 3000 phage/cell compared to ~25-100 phage/cell in other phages. To deconstruct the N4 LIN pathway for future applications, we used RNA sequencing to identify all molecular players involved. Infected samples of wildtype N4 and a rapid lyser variant that does not exhibit LIN were collected to capture the important stages of the N4 lysis program. These transcriptomic data show the expression of an unannotated open reading frame we named gp75 which has nonsense mutations in all sequenced rapid lyser lines but not the wildtype. Overexpression of wildtype gp75 in cells infected by the N4 rapid lyser do not undergo rapid lysis as compared to cells with no gp75 expression. This implies a strong association between LIN and gp75. Ongoing studies will delineate the function of gp75 and examine the role of differentially expressed host & N4 genes.

GSP 14

Ribosomal protein bL27: A key regulator of trans-translation efficiency and antibiotic sensitivity

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Trans-translation is a novel antibiotic target because it is essential in most bacteria and is not present in animals. *Trans*-translation involves rescuing ribosome stalled at the 3' end of mRNAs lacking stop codons, employing a ribonucleoprotein complex consisting of tmRNA and SmpB. A family of acylaminooxadiazoles that inhibit *trans*-translation have potent antibiotic activity. Previous studies using single-particle cryo-EM on non-stop ribosomes revealed that the binding of acylaminooxadiazoles to a distinct site close to the peptidyl-transfer center leads to significant changes in the conformation of the N-terminus of bL27. This observation suggests an innovative mechanism for the targeted inhibition of *trans*-translation by these compounds. Here, we investigate the features of bL27 that are required for inhibition of *trans*-translation by these molecules and ribosome function.

Site directed mutagenesis analysis on the N-terminal sequence of bL27 showed that changes in positions H3, K4, and K5 result in hypersensitivity to trans-translation inhibitors. Furthermore, deletion of bL27 confers greater sensitivity to acylaminooxodiazole-based trans-translation inhibitors, suggesting a critical role for bL27 in ribosome rescue.

In vitro trans-translation experiments with ribosomes lacking bL27 showed increased trans-translation activity making bL27 the only ribosomal protein known to contribute to trans-translation in vitro. Our findings illuminate the

significance of bL27 in bacterial ribosome rescue pathways and provide insights into the mechanism of acylaminooxodiazole-based trans-translation inhibitors.

GSP 15

Envelope stress responses functionally coordinate to maintain cell homeostasis in *Escherichia coli*.

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The envelope stress responses (ESRs) are essential pathways that maintain cell integrity and protect bacteria from antibiotics. Two critical ESRs that monitor outer membrane integrity in *Escherichia coli* are the Regulator of Capsule Synthesis (Rcs) and the extracytoplasmic σE response. Rcs and σE survey lipopolysaccharide (LPS) and outer membrane protein (OMP) biogenesis and are often induced under the same conditions. My project investigates potential regulatory feedback between them. Using σE-dependent transcriptional fusions, I showed that Rcs stimulates σE activity. Next, I focused on the mechanism underlying this stimulation. σE activity is regulated post-translationally by the anti-σ factor RseA, which is degraded in response to OMP defects, allowing free σE to regulate gene expression. My genetic epistasis analysis demonstrated that not only Rcs acts independently of RseA but can also act synergistically with the RseA degradation pathway to stimulate σE activity.
σE - encoding gene, *rpoE*, forms an operon with *rseA*, and their expression is highly regulated. Previous studies implicated RcsB in controlling one of the minor *rpoE* promoters. I generated several transcriptional reporter fusions and showed that when Rcs is strongly activated, it indeed upregulates the expression of the *rpoE-rseA* operon. We hypothesize that by doing so, Rcs increases the amount of the σE protein that is made, while the RseA degradation pathway increases the fraction that is active, enabling a synergistic response. As LPS and OMPs are the major components of the outer membrane, this Rcs/σE regulatory feedback may help coordinate outer membrane homeostasis.

GSP 16

Isolation and characterization of bacteriophages infecting *Pseudomonas aeruginosa*: exploring ecological diversity and therapeutic potential.

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Bacteriophages, viruses that specifically infect bacteria, are emerging as promising therapeutic agents against antibiotic-resistant pathogens. These natural predators offer a potential alternative or complement to traditional antibiotics, addressing the growing global health threat of antimicrobial resistance. Their ability to target specific bacterial species while leaving beneficial microbes unharmed makes them attractive for therapeutic applications. *Pseudomonas aeruginosa* is a notorious opportunistic pathogen, frequently associated with hospital-acquired infections and known for its remarkable ability to develop antibiotic resistance. The increasing prevalence of multidrug-resistant *P. aeruginosa* strains has led to urgent interest in alternative treatment strategies, including phage therapy. Our research has successfully isolated and characterized six phages capable of infecting *P. aeruginosa* strains from playa lakes in Lubbock, TX. Specifically, we isolated phages BL1, BL2, and BL3 for strain PAO1, and BL4, BL6, and BL7 for strain PAK. SPAdes assembly metrics confirmed single large contigs (>5000bp) for each bacteriophage. Genomic analysis revealed that these lytic bacteriophages belong to an unclassified species within the genus *Pbunavirus* of the *Caudoviricetes* class. Initial genomic analysis using CheckV did not identify any virulence or antibiotic resistance genes, which is promising for potential therapeutic applications. Yet further comprehensive studies would be necessary to conclusively determine the safety profile of these phages. All data from this study, including genome sequences and raw sequencing reads, are publicly available in NCBI, SRA, and BioProject databases. This research enhances the understanding of phage diversity and emphasizes the potential of bacteriophages as therapeutic agents against antibiotic-resistant *P. aeruginosa* strains.

GSP 17

Addressing Microbial Corrosion During Spaceflight With AI-Driven Image Analysis

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The International Space Station's Water Recovery System (WRS), which recycles urine and cabin moisture, faces continuous microbial inoculation. This persistent microbial presence leads to biofilm formation on surfaces, posing dual risks: potential water supply contamination and microbial-induced corrosion (MIC) of critical system components. Our study investigated biofilm growth and MIC on stainless steel materials during two spaceflight experiments. Escherichia coli F11 and Pseudomonas aeruginosa PAO1 were inoculated in artificial urine medium, mimicking WRS fluid, with and without AgF disinfectant. CRS-21 involved 16, 24, and 117-day incubations, while CRS-29 used 4, 14, and 117-day periods with asynchronous ground controls also conducted. CRS-21 showed corrosion on both inoculated and uninoculated samples, indicating MIC and chemical corrosion. However, a glutamine contamination was discovered, leading to a second flight (CRS-29). While corrosion was less apparent in CRS-29, scanning electron microscopy revealed bacterial presence on all inoculated samples, regardless of AgF presence, suggesting biofilm formation as a key survival mechanism in spaceflight. Key variables between missions included different stainless steel coupon batches and the CRS-21 contamination, resulting in divergent outcomes. CRS-21 exhibited readily detectable corrosion, while CRS-29 showed markedly less. These findings highlight the critical influence of material composition and environmental factors on MIC in spaceflight conditions, emphasizing the need for robust mitigation strategies in space-based water systems

GSP 18

Inhibitors of an extra-cytoplasmic function (ECF) sigma factor, σE as anti-infectives and antibiotics against Gram-negative pathogens

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The rise of antimicrobial resistance underscores the need for new antibiotics and anti-infectives that target pathogens effectively and specifically. Bacterial transcription is a potential, yet under-exploited target, with only two transcription-targeted antibiotic classes available. This project focuses on developing anti-infectives that target an alternative sigma factor, σ^E that is essential for the transcription of genes maintaining cell membrane composition. Since σ^E is conserved and essential for either viability or virulence in multiple Gram-negative pathogens, its inhibitors can function as either traditional antibiotics or anti-infectives that have little selective pressure and potentially lower resistance rate.

A high-throughput cell-based screening, in collaboration with GSK, was carried out to identify σ^E inhibitors. A preliminary Structure-activity relationship (SAR) was established from 47 analogs of the primary hit, with the most promising analog, KKL-39731 being the initial lead for subsequent SAR analysis. Bacterial growth inhibition and killing abilities of new KKL-39731 analogs were determined by measuring MIC and MBC against a panel of Gram-negative pathogens. Their on-target activity was validated by *in vitro* single-round transcription assay using *rybB* gene under the control of a σ^E-dependent promoter and *in vivo* fluorescence-based reporter assay using an engineered *Escherichia coli* strain expressing *gfp* gene downstream of a σ^E-dependent promoter. Microscale thermophoresis was used to further validate and quantify the effect of active compounds on the binding of σ^E to RNA polymerase and DNA promoter. Taken together, these assays not only demonstrate the use of σ^E as a promising antibiotic target but also enhance our understanding of its activity.

GSP 19

Phylogenomic framework for Escherichia coli of serotype O118: Virulence gene prevalence and pathovar boundaries informed by the comprehensive profiling of 359 genomes

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Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are of increasing public health concern. Circulating among these are STEC O118 that carry a potent phage borne-cytotoxin, often found alongside the locus of enterocyte effacement (LEE) pathogenicity island. This pathogroup causes severe human gastrointestinal disease that may progress to kidney failure. The objective of this study was to provide a serogroup-scale analysis to delineate both the phylogenomic and pathovar boundaries in O118 *E. coli*. For this study, all currently publicly available O118 genomes were retrieved from NCBI GenBank and profiled, along with two laboratory sequences genomes. A total of 359 genomes were imported into Ridom SeqSphere+ for targeted and core genome MLST, and the global gene reservoir was determined and classified with Roary and Scoary. Virulence and antibiotic resistance genes were identified using Virulence Factor Database and ResFinder. Carried prophages, genomic islands, and IS elements

were detected with PHASTER, IslandViewer4, and ISEscan, respectively. Pangenome analyses indicated a high degree of genome plasticity and allowed us to further gain insights into the common, shared and strain-specific virulence and resistance traits associated with the different STEC and non-STEC pathogroups. The established high-resolution cgMLST-phylogeny, inferred from the shared gene set of 4,160 genes, revealed a phylogenetic clustering based on H-type, along with a distinct grouping of STEC and non-STEC isolates. The STEC-pathogroups feature six toxin subalelles (*stx*$_{1a,1c,2a,2b,2c,2f}$). The vast majority are LEE+ isolates carrying intimins *eae*-I, -β, -ε; which show a strong correlation between the catalogued H- and respective intimin-subtype.

GSP 20

Long chain fatty acids promote *Candida albicans* gut colonization

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The human fungal pathobiont *Candida albicans* is a facultative anaerobe that resides in our digestive tract. *C. albicans* usage of various carbon and nitrogen sources have primarily been investigated under aerobic conditions. Consequently, the nutrients that fuel *C. albicans* growth in environments predominantly devoid of oxygen like the large intestine remain undefined. Here, we report that under anaerobic conditions, oleic acid supports enhanced *C. albicans* growth compared to common carbon sources such as glucose or lactate. Consistent with this finding, supplementation of rodent diet with high-oleic safflower oil resulted in enhanced *C. albicans* murine gut colonization. Oleic acid is one of the most abundant long chain fatty acids in nature. Diet, bacteria, and the host itself are sources of oleic acid in the digestive tract. We show that in the presence of oleic acid in the culture medium, conspicuous lipid droplets accumulate in the yeast cell cytoplasm, and that this process is largely independent of b-oxidation. Finally, RNA-Seq experiments revealed a large set of *C. albicans* transcripts upregulated in the presence of oleic acid under anaerobic conditions. Our findings suggest that long chain fatty acids may modulate the abundance of *C. albicans* in the mammalian gut.

GSP 21

Elucidating resistance mechanisms to the BAM complex inhibitors.

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Gram-negative bacteria present a significant problem for antimicrobial therapies due to the presence of an outer membrane. The outer membrane restricts the entry of many compounds, including antibiotics. One strategy to overcome the outer membrane permeability barrier is to develop inhibitors that target essential proteins on the bacterial surface. One such protein is BamA, a component of the β-barrel assembly machinery, known as the BAM complex that folds and inserts outer membrane proteins. BamA emerged as an attractive target, and its small molecule, peptide, and antibody inhibitors have been reported.
MRL-494 is a small molecule inhibitor of BamA, but mechanisms of action and resistance remain poorly understood. We isolated several novel MRL-494 resistant mutations targeting genes outside the Bam complex. These mutations caused LPS structure modification either by directly targeting an LPS biosynthesis enzyme LpxM or regulatory PmrAB and QseBC pathways. The PmrAB pathway has been well-studied for controlling EptA and ArnT enzymes that modify the lipid A portion of LPS, reducing its overall negative charge. I showed that deleting the *eptA* and *arnT* genes attenuated MRL-494 resistance in strains with constitutively active PmrAB pathway. We are currently investigating whether LPS structure influences Bam complex activity or helps cells overcome OMP assembly inhibition by modifying outer membrane properties.

GS Bacterial Pathogenesis:

GSP 22

Decoding In-Host Transcriptomic Adaptations of Staphylococcus aureus in Response to Mammalian Defensins

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Staphylococcus aureus, armed with drug resistance, looms as a global menace. The skin's innate immune system, bolstered by antimicrobial peptides (AMPs) such as defensins, swiftly responds to *S. aureus* infections, directly killing bacteria while also signaling to neutrophils to gather and eliminate bacteria. Our previous research revealed that mice lacking the *Def*ensin gene cluster (*Def* cKO mice) showed compromised anti-*S. aureus* immunity, with higher bacterial burden and impaired neutrophil response via the Mrgpr2a/b receptor. *S. aureus* encountering stress posed by the host immune system undergoes internal changes. Defensins stress *S. aureus* by causing membrane and cell wall damage, while the bacterium senses stress via two component systems and adapts through rapid gene expression changes. We aim to understand how *S. aureus* respond to mammalian defensin in vivo. To do this, we infect WT and *Def* cKO mice with *S. aureus*, isolate bacterial RNA from the infected skin, and compare bacterial transcriptome between the two conditions. We hypothesize that the presence of defensins and defensins receptors on neutrophils alter bacterial gene expression. Mapping the *S. aureus* gene regulatory network in response to AMPs and neutrophil stress in vivo will enhance the development of targeted immunotherapies for *S. aureus* infections.

GSP 23

Nafcillin Resistance Drives Azithromycin Sensitivity in MRSA

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The emergence of multidrug-resistant pathogens poses a significant clinical challenge, necessitating novel strategies to combat the evolution of antibiotic resistance. Collateral sensitivity or the “seesaw effect” is an intriguing and rare evolutionary trade-off within which resistance to one antibiotic leads to increased susceptibility to another. In this study, we discovered a seesaw effect between azithromycin and nafcillin in *Staphylococcus aureus*. To better understand the novel seesaw interaction, we evolved methicillin-resistant *Staphylococcus aureus* (MRSA) using two strategies: tolerance adaptive laboratory evolution (TALE), which imposes a weaker evolutionary pressure but maintains continuous exponential growth, and the resistance adaptive laboratory evolution (RALE), which imposes a stronger selection pressure yet allows entry into stationary phase. Remarkably, the TALE strains evolved to 128-fold nafcillin resistance and revealed up to 256-fold increased azithromycin sensitivity. The RALE strains, on the other hand, developed 1024-fold nafcillin resistance and exhibited only a 4-fold increase in azithromycin sensitivity. Further investigation revealed that the RALE strains have a stable small colony variant (SCV) phenotype, a phenotype often linked to persistent and relapsing staphylococcal infections. SCVs have extensively challenged efforts to control *S. aureus* infections, due to their spontaneous origination and ability to resist multiple antibiotics. This study can help us understand the link between SCV formation and resistance evolution, offering potential therapeutic strategies for exploiting vulnerabilities in multidrug-resistant microorganisms.

GSP 24

Examination of Campylobacter and Enterococcus Interactions in an Animal Infection Model

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Galleria mellonella, the Greater Wax Moth, has been used as a relatively simple bacterial infection model due to its animal immune system and physiology. *G. mellonella* has innate responses to bacteria, including phagocytes. *Campylobacter jejuni* is a common food-borne pathogen found mostly in retail meats, with the CDC estimating 1.5 million annual cases of campylobacteriosis in the US. These microaerobic Gram-negative bacteria can cause diarrhea and cramping, and without treatment can lead to irritable bowel syndrome (IBS) or Guillain-Barre syndrome. *C. jejuni* was introduced into the worms orally or via bodily injection at four different doses, to observe the pathogenesis of *C. jejuni* and the immune response from *G. mellonella*. Worm coloration (an immune marker) and some lethality were observed. During foodborne infections, *C. jejuni* is constantly exposed to other species of bacteria, both in food and in the gut. *Enterococcus* is a normal gut resident in humans, and other studies have shown it can enhance infections with *E. coli* or *C. difficile* or conversely act as a probiotic to protect against diarrhea and gut inflammation. In future work, we plan to use our developed model to determine if *Enterococcus* can increase the virulence of *C. jejuni* in the gut, and if so, begin to explore potential mechanisms.

GSP 25

Using GWAS to investigate how antibiotic resistance evolves in *Pseudomonas aeruginosa* during Cystic Fibrosis

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Cystic fibrosis (CF) is a fatal disease caused by a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which codes for a channel responsible for chloride and bicarbonate movement. Mutation in CFTR leads to abrogation of chloride and bicarbonate channels, dissipating the presence of chloride and bicarbonate in the airway. *Pseudomonas aeruginosa* is a gram-negative pathogen that colonizes CF patients. As infection progresses, and lung function declines, P. *aeruginosa* evolves high levels of antibiotic resistance and becomes progressively harder to kill. However, how bacteria evolve antibiotic resistance (AR) in the conditions of the CF lung is unclear. The following study proposes using a GWAS of CF patients versus healthy controls to understand how antibiotic resistance evolves in CF. So far, phenotypic data was collected from the CF foundation, Bv-Brc, and NCBI. Both CF and non-CF Biosample IDs were filtered based on presence of at least one CF and AR type entry, and non-CF Biosample IDs were generated using filtering based on random counting. The entrez package in BioPython was used to pull down genomic data from the NCBI server base using each genome name's Biosample ID. The genomes were saved to a FASTA file for future GWAS analysis. The expected results of this study are that the GWAS will reveal novel interactions between loci associated with AR. Future studies will focus on confirming identified CF AR mutations.

GSP 26

Colistin and Meropenem Seesaw Interactions in *Acinetobacter baumannii*

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Antibiotic resistance is evolving at a rapid rate, presenting a growing critical health threat. *Acinetobacter baumannii*, notorious even among its fellow ESKAPE pathogens for its aggressive ability to evolve multidrug resistance, exemplifies this trend as we see increasing emergence of resistance to even our drugs of last resort. A promising strategy for combatting this unrestrained evolution is exploiting collateral susceptibilities, also called "seesaw interactions." Seesaw interactions are a phenomenon in which increasing resistance to one antibiotic causes a decrease in resistance to another antibiotic. To test this phenomenon, we used adaptive laboratory evolution (ALE) to generate multiple lineages of colistin-resistant A. baumannii and screened for seesaw interactions guided by a prediction model based on published minimum inhibitory concentration (MIC) data. Remarkably, we found some level of collateral sensitivity with many of these drugs. Most notably, we observed up to a 256-fold reduction in meropenem MICs, and all 12 end-point strains had been driven below the CLSI-defined resistance threshold. Additionally, while high level of colistin resistance could develop within 3-4 days, the presence of sub-inhibitory meropenem prevented the evolution of colistin resistance until the end of the experiment at 14 days. Ongoing genomic and physiological analysis aims to elucidate the underlying mechanisms associated with these observed shifts in antibiotic susceptibilities. Utilizing antibiotic seesaw interactions has the potential to slow the progression of resistance long-term and preserve the effectiveness of our current antibiotics.

GSP 27

Competitive Fitness of Asymptomatic Bacteriuria *E. coli* Strain 83972 Against Uropathogens in Human Urine

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Urinary tract infection (UTI) is one of the most common bacterial infections worldwide. The main causative agent of UTI is uropathogenic *Escherichia coli* (UPEC). There is an immediate need for novel prophylactic and treatment strategies against UTI because of the increasing incidence of antimicrobial resistance among uropathogens. ABU 83972, an asymptomatic bacteriuria-causing *E. coli* strain, prevents UTI by suppressing the colonization of UPEC. However, the nature of competition and growth repression of UPEC by ABU 83972 is unclear and is the subject of our investigation. Here, we characterized the growth kinetics of ABU 83972 and uropathogens in human urine and laboratory media. Next, we performed a series of competitive co-culture experiments where ABU 83972 and uropathogens were inoculated at a 1:1 ratio in human urine and in various media, and their relative abundance was determined. In human urine, ABU 83972 outcompeted UPEC and additional uropathogens, reaching up to 90% of the total population after 24 hours of incubation. In contrast, UPEC outcompeted ABU 83972 in LB and M9 minimal media and exhibited superior colonization than ABU 83972 in the mouse urinary bladder. Since engineered living materials (ELMs) can be used to retain an organism of interest in a particular location, we developed ABU 83972-containing ELMs that effectively outcompeted UPEC in human urine. In summary, our work establishes that ABU

83972 outcompetes UPEC in a milieu- and cell-density-dependent manner, highlighting the importance of the metabolites and nutrients found in the human urine as determinants of the competitive fitness of ABU 83972.

GSP 28

Staphylococcus aureus in Orthopedic Device-related Infection Biofilm Models

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The rise of antimicrobial resistant (AMR) infections is a growing concern in the treatment of osteomyelitis (OM) and orthopaedic device-related infections (ODRIs). *Staphylococcus aureus* is responsible for 33-43% of these infections due to its prevalence in hospital settings, its numerous antibiotic-resistant variants, and its ability to form biofilms. Bacteriophage (phage) therapy is now being considered as a treatment for AMR OM/ODRIs. We are investigating the use of phage encapsulated into microspheres for local delivery of phage to the site of AMR OM/ODRIs infections. We determined that the anti-staphylococcal phage K effectively lyses the *S. aureus* clinical isolate UAMS-1 growing in liquid culture, on agar plates, and as an *in vitro* biofilm. Our UAMS-1 *in vitro* biofilm model closely mimics the bone infection environment. UAMS-1 produces biofilms on polymethylmethacrylate (PMMA, bone cement) discs in synthetic synovial fluid in static 24-well plates. After three days, we can expose these biofilms for 24 hours to purified phage (10^5 and 10^8) or phage eluted from microspheres (10^5). The effect of phage treatment on biofilm growth can be quantitated by confocal imaging of discs exposed to BacLight Live/Dead stain (Invitrogen) and determining viable and direct counts of the disc sonicates. Our image analysis is in good agreement with the cell counts indicating that untreated 4-day biofilms contain about 10^7 cells per 0.5 cm disc. Further studies are warranted to gauge the effectiveness of the microspheres against *S. aureus in vitro* biofilms and in an *in vivo* animal model.

GSP 29

Investigating the interplay between diet and the gut microbiome in the pathogenesis of necrotizing enterocolitis

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Necrotizing enterocolitis (NEC) is the leading cause of gastrointestinal-related deaths in premature infants. Although the exact mechanisms of the disease are not fully understood, certain risk factors like prematurity, diet, and gut microbiome composition have been identified. Notably, breastmilk has been found to significantly reduce the incidence of NEC compared to infant formula. However, the specific mechanisms by which breastmilk or formula influences NEC pathogenesis remain unclear. This research project aims to explore how the interaction between diet and the gut microbiome impacts susceptibility to NEC. Studies utilizing a preterm piglet model have shown that piglets fed with commercially available infant formula exhibit a higher incidence of NEC compared to those fed with donor human milk (DHM). Whole genome sequencing of the gut microbiome revealed that Clostridium perfringens colonization was more prevalent in formula-fed piglets, correlating with greater disease severity. This suggests a potential role of C. perfringens in the development and progression of NEC. Interestingly, C. perfringens isolates from NEC-afflicted piglets displayed higher growth rates and increased levels of the toxin perfringolysin O when cultured in formula compared to DHM. Moreover, piglet and infant isolates grow more densely in maltodextrin, the primary carbohydrate in formula, than in lactose, the dominant sugar in breast milk. These results suggest that the composition of formula may favor the growth and virulence of harmful gut microbes associated with NEC. Future research will investigate how dietary components influence the abundance of pathobionts and how DHM protects the preterm gut from bacterial injury and NEC.

GSP 30

Connection between *clpX* and *msrB* and increased sensitivity to cell wall antibiotics in *Bacillus anthracis* Sterne

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Our lab has identified that ClpX, a regulatory ATPase, plays a role in resistance to cell-envelope targeting antibiotics and is critical for virulence in *B. anthracis* Sterne. ClpX works with a ClpP subunit to form the ClpXP protease, which

maintains overall protein homeostasis. ClpX recognizes and unfolds proteins, then passes them to ClpP for degradation. However, it is unlikely that ClpX is directly mediating these effects; instead, these effects are likely due to the dysregulation of the protein network maintained by ClpXP. Previously, we conducted a microarray to identify genes differentially expressed between wild-type *B. anthracis* Sterne and a *ΔclpX* strain and found 119 dysregulated genes. One of the identified genes, *msrB*, is methionine sulfoxide reductase, an antioxidant enzyme that restores functionality to oxidized methionine residues. Primarily, *msrB* has been linked to reactive oxygen species (ROS) tolerance in other species of bacteria. In *S. aureus*, *msrB* expression was induced upon exposure to oxacillin, indicating a potential connection between MsrB and cell wall-targeting antibiotics. In *B. anthracis* Sterne, loss of *msrB* increases susceptibility to penicillin and vancomycin. However, this phenotype is not seen with cell-membrane targeting agents, suggesting that the role of *msrB* in antimicrobial resistance may be limited to cell-wall active antibiotics. Additionally, as MsrB is an antioxidant enzyme, we are exploring its role in resistance to ROS, such as H2O2, sodium hypochlorite, and paraquat. Our research provides further information on how the ClpXP protease could mediate resistance to different antibacterial agents through downstream proteins such as MsrB.

GSP 31

Impact of Bicarbonate on *Pseudomonas aeruginosa* virulence and host immune interactions in Cystic Fibrosis

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Cystic Fibrosis (CF) is an autosomal recessive genetic disorder characterized by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene, leading to impaired efflux of chloride and bicarbonate (HCO_3^-) ions. CF predisposes patients to severe *Pseudomonas aeruginosa* infections that develop antibiotic resistance over time and ultimately compromise their lung function. We have previously found that HCO_3^- can improve antibiotic susceptibility against resistant pathogens. However, as host immune cells also respond to HCO_3^-, it is unknown how HCO_3 impacts combined host-pathogen interactions. We have performed antibiotic susceptibility testing with HCO_3^- and observed differential responses of various classes of antibiotics against *P. aeruginosa.* Then we conducted macrophage infection assay with THP-1 cells against *P. aeruginosa* clinical isolate, P4. Surprisingly, HCO_3^- was found to enhance the bacterial survival. We also observed that P4 has shown increased survival when it is either pre-incubated with HCO_3^- or HCO_3^- is present during the infection. Survival of P4 is found to be lower when there is no HCO_3^- in the bacterial and macrophage cultures. These findings suggest that HCO_3^- impacts both the bacteria and macrophage. By elucidating the intricate relationship between HCO_3^-, bacterial virulence, and host immune responses, we aim to provide valuable insights for improving patient outcomes and addressing the challenges posed by antibiotic-resistant pathogens in CF.

GSP 32

Examining how bicarbonate resistance fuels virulence in *Acinetobacter baumannii*

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Acinetobacter baumannii, a highly antibiotic resistant pathogen, is considered a high priority pathogen according to the WHO and a critical threat by the CDC. While there are many virulence factors associated with this gram negative bacterial pathogen, its survival mechanism in the presence of bicarbonate remains poorly understood. Bicarbonate typically has an inhibitory effect on bacterial growth, making resistance to it a related factor of pathogenicity. This study therefore investigates the relationship between bicarbonate resistance and virulence in multiple multi-drug resistant *A. baumannii* strains. We selected a laboratory strain (AB5075), two clinical isolates with contrasting antibiotic susceptibility profiles (pan-sensitive [PS] and pan-resistant [PR]), and a colistin resistant evolved strain. We observed the PR strain was resistant to a majority of antibiotics in our in vitro testing, while the PS strain was resistant to only colistin and carbapenems. Despite this, the PR strain had reduced virulence in mice while the PS strain was highly virulent. Various in vitro tests were conducted on the strains of interest to better understand these virulence differences. Surprisingly, the majority of in vitro tests indicated no significant difference between the strains. However, analysis of growth kinetics showcased clear differences of bacterial growth in media containing bicarbonate. Our data indicates a noticeable relationship between bicarbonate resistance and virulence in multiple colistin resistant *A. baumannii* strains. With additional research we hope to gain a better mechanistic understanding between virulence and bicarbonate resistance in *A. baumannii*, potentially leading to novel approaches in combatting this pathogen.

GSP 33

Novel Biofilm Mutations Promote Minocycline Resistance in *Acinetobacter baumannii*

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Acinetobacter baumannii is a gram-negative human pathogen that poses significant challenges in healthcare settings due to its ability to acquire multidrug resistance. Minocycline is one of the few remaining effective antibiotics for treating *A. baumannii* infections, yet resistance is beginning to emerge. Understanding the mechanisms underlying minocycline resistance is essential to preserve the antibiotics efficacy in the clinic. In this study, we utilized a machine learning prediction model to identify genes associated with minocycline resistance. Based on the predictions we selected 36 genes for further minocycline sensitivity analysis. We found that one of the mutants, *ruvB*, was 32-fold more resistant to minocycline than wild type *A. baumannii* AB5075. The Ruv gene system, consisting of *ruvA*, *ruvB*, and *ruvC*, is typically involved in DNA repair and recombination processes. MIC tests were conducted on all three mutants (ruv*A*, ruv*B*, and ruv*C*) using Doxycycline and Tetracycline, and intriguingly, only the Ruv*B* mutant demonstrated resistance to these antimicrobial agents. Prompted by the initial observation of high minocycline resistance in the ruv*B* mutant during screening, further evaluation revealed its pronounced ability to form robust biofilms. Interestingly, there was no difference in biofilm formation with either the *ruvA* or *ruvC* mutant in *A. baumannii* AB5075 strain. genetic studies in a different *A. baumannii* strain, ATCC 19606, also confirmed the same phenotype. This study has identified a novel mechanism of broad antibiotic resistance in *A. baumannii* through disruptions in the RuvABC DNA repair system.

GSP 34

A Reverse Genetic Screen Elucidates the Mechanisms of Anaerobic Copper Toxicity in *E.coli*

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Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, afflicting millions of people annually, and thus, are a leading cause of antibiotic prescription. UTIs can be caused by a diverse range of pathogens; however, the overwhelming majority of these infections emerge as a result of Uropathogenic *E. coli* (UPEC). UPEC is exposed to host-derived copper under low oxygen conditions during UTI. This study seeks to elucidate the mechanisms utilized by UPEC to overcome copper toxicity under anaerobic conditions. We performed a copper sensitivity screen with an *E. coli* single-gene knockout library under anaerobic and aerobic conditions in vitro. Our reverse genetic screen has identified 264 genes utilized by *E. coli* to survive under anaerobic copper stress. Specifically, loss of genes regulating metabolism and trace mineral homeostasis, including molybdenum and sulfur, were more sensitive to copper. By investigating the effect of a host immune effector against *E. coli* under infection-relevant conditions, our findings are expected to identify targets for the future development of novel treatment strategies against UPEC.

GSP 35

Redefining Antibiotic Resistance in *Acinetobacter baumannii*

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Acinetobacter baumannii infections represent a significant public health challenge due to the pathogen's rising resistance to antibiotics, particularly that last-line of defense antibiotic: polymyxin E (colistin). While current colistin resistance rates in *A. baumannii* remain relatively low, the underlying mechanisms of resistance are not well understood. In this study, we utilized a machine learning model trained on clinical *A. baumannii* isolates to identify 31 genes associated with colistin resistance. These genes encompass diverse functions, including membrane biosynthesis, iron transport, transcription regulation, DNA repair, and ATP metabolism. To evaluate the role of these genes in colistin resistance, we employed the Manoil AB5075 transposon library and conducted standardized antimicrobial susceptibility testing on each mutant. Despite no significant changes in Minimum Inhibitory Concentration (MIC) values among the 31 mutants, further analyses revealed intriguing adaptive fitness phenotypes in response to colistin exposure. Notably, approximately 68% of the mutants exhibited reduced fitness, while 13% showed enhanced fitness under sub-inhibitory colistin concentrations. Additionally, most mutants displayed notable alterations in membrane properties, biofilm formation, efflux pump activity, and oxidative stress response when challenged with colistin. These findings underscore the limitations of relying solely on MIC values to gauge colistin resistance in *A. baumannii*, suggesting that routine susceptibility testing may not fully capture the pathogen's adaptive responses. Ultimately, our study highlights the necessity for more comprehensive approaches beyond MIC measurements to accurately characterize colistin resistance in *A. baumannii*, prompting the need to reevaluate how antibiotic resistance is defined in bacterial pathogens.

GS Medical Microbiology:

GSP 36

Increased Risk of Dementia Associated with Herpes Simplex Virus Infections: Evidence from a Retrospective Cohort Study Using U.S. Electronic Health Records

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Alzheimer's disease is the most common age-related dementia. Recent compelling evidence from previous retrospective electronic health record (EHR) studies suggests that Herpes Simplex Virus (HSV) infections may be a risk factor for developing dementia. However, no age and propensity score-matched studies have been published in a United States general population cohort study to date. We aimed to identify whether HSV infection shows a significantly increased risk of the development of dementia in a sizable and heterogeneous cohort. We investigated whether Herpes Simplex Virus Type 1 (HSV1), Herpes Simplex Virus Type 2 (HSV2), or coinfections with both serotypes pose a greater risk of developing dementia across different biological sexes and racial groups. EHR from patients with a history of HSV or specific serotypes (HSV1 or HSV2) infection were selected for analysis. These records were compared to a propensity-matched control group and analyzed for hazard and odds ratios through TriNetX. There was a significant difference in dementia incidence in the HSV-infected group versus the control. Individuals with a history of HSV, HSV1, HSV2, and coinfection all showed a significant risk of developing dementia compared to controls. Males with HSV2 are at a higher risk of dementia outcome than females with HSV2. While consistent with previous reports, these findings are the first to establish a higher risk of developing dementia in patients who have any HSV diagnosis using a nationwide, population-based matched cohort study in the United States.

GSP 37

Elucidating the role of Fatty Acid Binding Proteins (FABPs) in KSHV Replication and Maximal Infectious Virion Production

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Kaposi’s sarcoma-associated herpesvirus (KSHV), a double-stranded enveloped DNA virus, is the etiological agent of Kaposi’s Sarcoma (KS); an endothelial cell-based tumor, and the most common cancer in HIV-infected individuals. Currently, there is no treatment for KSHV therefore, it is vital to identify the underlying mechanisms of viral infection. KSHV infection of endothelial cells results in morphological, metabolic, lifespan, and gene expression changes to facilitate virion production. In a previous study, the fatty acid binding protein (FABP) genes were observed to be differentially regulated by KSHV in Burkitt Lymphoma cells (BJAB). Fatty acid binding proteins are cytosolic proteins that play a central role as lipid chaperones, trafficking fatty acids to specific cellular compartments. Nevertheless, the role of FABPs in lytic KSHV infected endothelial cells has not been explored. Preliminary data in iSLK BAC16 cells, a KSHV reporter cell line and model of latent and lytic infection, show increased FABP2 and FABP4 mRNA expression levels post lytic induction. **Therefore, I hypothesize that the differential expression of FABPs during lytic KSHV infection is required for KSHV replication and maximal infectious virion production.** To test this hypothesis, I will employ RT-qPCR to measure mRNA expression levels of early and late lytic genes after siRNA-mediated knockdown of FABP2/4. Furthermore, I will determine intracellular and extracellular maximal infectious virion production following FABP2/4 knockdown in iSLK BAC16 cells through viral titrations in endothelial cells. This experimental approach of studying FABPs will infer to the role of FABP2 and FABP4 in facilitating viral infection and pathogenesis.

GSP 38

DEVELOPING A NOVEL MURINE MODEL OF POLYTRAUMA-ASSOCIATED MUCORMYCOSIS

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Polytraumatic injury (life-threatening injury to multiple body regions) results in systemic immunological dysregulation that predisposes a patient to serious invasive fungal infection. Polytrauma-associated infection by environmental molds of the order Mucorales is most often seen after natural disasters or combat injuries, even occurring in otherwise

healthy, young patients. There are currently few preclinical animal models of polytrauma-associated mucormycosis, which limits our understanding of host response and fungal pathogenesis and restricts our ability to functionally test novel therapeutics. We have established a murine model of polytrauma-associated mucormycosis in healthy B57BL/6J mice with no prior immunosuppression. In this model, mice are first subjected to multilateral scalding water burns followed by a skeletal muscle cryoinjury on an unburned flank. The cryoinjury is then contaminated directly with *Mucor* spores. Mice exposed to polytraumatic injury develop increased fungal growth at the wound site compared to mice exposed to cryoinjury only. Current work includes quantifying fungal growth and dissemination via qPCR on organ homogenates. In the future, we plan to use this model to identify and target critical host responses that contribute to mucormycosis infection pathology and detrimental outcome. Ultimately, this model will allow us to test novel therapeutics in an animal model that more closely reflects the severe response to trauma.

GSP 39

Characterizing the role of the Scp160 SESA Complex Component in Regulation of *Candida albicans* Virulence Properties

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Candida albicans is an opportunistic fungal pathogen that causes severe disease in immunocompromised individuals such as HIV and cancer patients. *C. albicans* can undergo a reversible morphological transition from yeast cells to filamentous hyphae, which is required for virulence. Filamentation of *C. albicans* is regulated by both transcriptional and post-transcriptional mechanisms. Scp160, a mRNA-binding protein that has been studied in the model yeast *Saccharomyces cerevisiae*, is a component of the SESA complex, which also contains Smy2, Eap1, and Asc1. This complex inhibits the translation of specific mRNAs, such as POM34, under conditions in which spindle pole body (SPB) is defective. However, the Scp160 ortholog in *C. albicans* has not been characterized and it is unclear whether this protein has a conserved function. Preliminary studies suggest that other SESA complex components in *C. albicans*, such as Asc1 and orf19.7034, the ortholog of Eap1, regulate virulence properties such as filamentation and stress responses. Our current study suggests that *C. albicans* scp160 deletion mutants are resistant to cell membrane and cell wall stressors. Additionally, preliminary data has shown that the scp160Δ /Δ mutant is hyper-filamentous under certain filament-inducing conditions. Characterizing the novel role of Scp160 in *C. albicans* and its interactions with Asc1 and orf19.7034 can provide evidence that the SESA complex is conserved, potentially leading to the development of an antifungal drug target. Findings of this study can also lay the foundation for future studies of Scp160 orthologs in other emerging human fungal pathogens such as *Candida auris* and *Candida glabrata*.

GSP 40

Immune correlates of memory T cells with vaccine-induced protection against fatal murine rickettsiosis

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Tick-borne rickettsioses (TBRs) can be life-threatening, but no licensed vaccines are available due to unknown mechanisms of host protection. We've demonstrated that single dose immunization with Rickettsia parkeri mutant 3A2, with a modified pLoxHimar transposon inserted into the gene encoding a phage integrase protein, confers complete protection against fatal R. parkeri rickettsiosis in mice. This study explored the role of memory T cells in the protection with this potential live-attenuated vaccine (LAV) against TBRs. R. parkeri 3A2 immunized mice were challenged with a lethal dose of wild type (WT) R. parkeri after three months immunization. Compared to mock immunized mice, 3A2-immunized mice showed complete bacterial clearance in their tissues upon lethal challenge with WT R. parkeri. The protection provided by 3A2 was associated with significantly increased frequencies of effector memory CD4 and CD8 T cells in the spleen, characterized by CD3+CD4+CD44highCD62Llow and CD3+CD8+CD44highCD62Llow, respectively. Ex vivo stimulation with phorbol 12-myristate-13-acetate (PMA) and ionomycin, splenocytes of 3A2 immunized mice prior to R. parkeri challenge showed significantly elevated percentages of IFN-gamma (+) TNF-alpha (+) CD44+ memory CD4+ T cells compared to those of control mice. Additionally, adoptively transfer of purified CD3+CD4+ T cells of 3A2-immunized WT C3H/HeN mice provided 50% protection to T- and B-cell-deficient C3H-scid mice against a lethal challenge with WT R. parkeri compared to mock-immunized controls. Our studies suggest that vaccine-induced protection against TBRs is associated with

memory CD4 T cells, particularly the expansion of effector memory T cells and memory CD4 T cells producing cytokines, including TNF-alpha and IFN-gamma.

GSP 41

Cathelicidin's impact on macrophage response during infection with Candida albicans

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Host defense peptides (HDPs) are short polypeptides that are both immunomodulatory and antimicrobial. Cathelicidin, named CRAMP and LL-37 in mice and humans respectively, is a HDP produced by epithelial cells and neutrophils in response to inflammation. In vivo, cathelicidin stimulates a variety of host responses including cellular activation, cytokine production, and differentiation. While cathelicidin's antimicrobial action on the fungal pathogen, Candida albicans, has been well described, the immunomodulatory impact during infection are less understood. We have shown that mice deficient in CRAMP (CRAMP KO) survive systemic fungal infection better than wild-type mice. This is associated with a reduced pro-inflammatory response in serum and major organs. Transcriptional analysis via qRT-PCR of bone derived macrophages suggests that CRAMP KO macrophages produce less inflammatory cytokines when infected with C. albicans. To determine the specific pathways impacted by cathelicidin during C. albicans infection we are performing whole-genome transcriptional sequencing on macrophages in vitro and organs in vivo. Future experiments include targeted blockade of known cathelicidin-receptor interactions as well as single-cell transcriptomics of in vivo macrophages. Ultimately, by delineating the specific role of cathelicidin in driving inflammation, these studies highlight the therapeutic potential of targeting cathelicidin to alleviate damaging host responses.

GSP 42

Exploring Regulatory Mechanisms of the Essential Immediate-Early M142 gene of Murine Cytomegalovirus

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Human cytomegalovirus infection worldwide occurs in between 50%-90% of people after which it enters a latent stage and persists for the lifetime of its host. In vulnerable populations, primary infection or reactivation from latency is responsible for significant medical complications. To improve our understanding of this ubiquitous virus, we investigated the regulation of the minor immediate-early gene, M142, which is essential for productive infection in the closely related murine cytomegalovirus. Initial tests supported the presence of one or more repressor binding sites within a large internal region of the gene's promoter. Excision of this region elevated reporter expression within two permissive cell lines maintained independently of active infection. These data support one or more conserved cellular factors act to repress the expression of this gene. Similar tests supported that one or more activator binding sites were present in a small distal portion of the promoter. A mutation in the core GGA triplet of a predicted Elk-1 site reduced affinity of unlabeled probes for nuclear fraction proteins during EMSA analysis and was subsequently incorporated in a reporter cassette. No significant reduction of reporter gene activity was detected in a neuroblastoma line; however, a significant elevation of reporter activity was detected in infected murine fibroblasts. These data support ELK-1 may play a repressive role in an otherwise powerful activator region. Our data reveal a complex regulatory schema in an essential minor immediate-early gene and may offer new targets to limit viral replication.

GSP 43

Unraveling the functional role of KSHV latent-host protein-protein interactions

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Kaposi's Sarcoma-Associated Herpesvirus (KSHV) is an oncogenic virus that causes Kaposi's Sarcoma. KSHV latent and lytic phases contribute to its pathogenesis. Viral-host protein interactions (PPIs) may remodel the host cell machinery for successful virus replication. However, the molecular mechanisms behind these interactions are still understudied. We hypothesize that KSHV latent-host PPIs are crucial for regulating cellular processes that contribute to KSHV infection. Therefore, we aim to determine the functional implications of KSHV latent-host PPIs. In this study, we evaluate the role of latent KSHV-host PPIs in either maintaining latency or supporting viral replication in a doxycycline (DOX)-inducible KSHV reporter cell line (iSLK.BAC16) through qPCR. Previous studies reported the

interaction of the latency-associated nuclear antigen, LANA, with the host death domain-associated protein (DAXX) and the DNA ligase 3 (LIG3). Our preliminary data shows increased mRNA expression levels of early (ORF45) and late (K8.1) lytic genes after DAXX knockdown in DOX-induced iSLK cells, suggesting that DAXX is required for latency maintenance. Conversely, LIG3-knockdown caused a decreased expression of these lytic genes, indicating that LIG3 might be necessary for viral reactivation in DOX-induced iSLK cells. Future studies are underway to understand the role of other KSHV latent-host PPIs in iSLK and endothelial cells. Understanding the mechanistic significance of these interactions in regulating the host endothelial cell response during infection is critical for developing potential treatment strategies.

GSP 44

Novel live-attenuated and subunit vaccines provide long-term protection against pneumonic plague

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Plague-causing Yersinia pestis is responsible for 3 major pandemics and a recent epidemic in Madagascar (9% fatality rate) where 77% of cases were pneumonic. The high mortality rate and limited antibiotic treatment window emphasize the need for vaccine prophylaxis; however, none are FDA approved. Our lab generated two live-attenuated triple mutants of Y. pestis, LMA and LMP, that were avirulent in a pneumonic mouse model while retaining immunogenicity. A trivalent subunit adenoviral vector-based Ad5-YFV vaccine was also developed, effective in mice and non-human primates. We performed detailed immunological characterization on LMA or LMP followed by Ad5-YFV in prime-boost or simultaneous strategies to assess long-term protective efficacy. Both approaches completely protected mice against lethal pneumonic challenge with virulent Y. pestis CO92. All vaccinated mice generated and maintained significant humoral immune responses based on IgG to F1V antigen compared to unvaccinated controls. Enhanced T cell activation, as well as memory B cell responses, were noted in vaccinated mice. All vaccination strategies increased percentages of T cells positive for IFNγ, IL-2, TNFα, IL-4, and IL-17 in mice. Vaccinated mice also produced significantly higher memory T cell responses compared to unvaccinated controls. In summary, vaccination with LMA or LMP and Ad5-YFV provided complete long-term protection and induced humoral and cellular immune responses. Vaccinated mice maintained immunological memory through production of memory B and T cells. These studies indicated that heterologous vaccination with LMA or LMP and Ad5-YFV are suitable approaches to prevent plague outbreaks in reactive and preventative scenarios by providing long-term protection.

GSP 45

Molecular Typing of Adenoviruses Associated with Respiratory Illness Among Humans and Poultry, Pakistan

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Adenoviruses (ADVs) have caused epidemics among both humans and poultry in Pakistan. While typically species-specific, recent evidence suggests ADV spillover infections occur more often than recognized. Between 2019 and 2021, we conducted surveillance for ADVs, collecting 1,705 swab specimens from humans, poultry, and livestock with signs of respiratory illness in Pakistan. Molecular evidence of ADVs infections was quite prevalent with 96 (8.8%) of 1,084 human oropharyngeal swabs and 15 (4%) of 385 poultry orotracheal swabs found positive. None of the 236 livestock samples were positive. Among humans, the odds ratio (OR) of ADV detection were greatest among participants with wheezing (OR=10.9, 95% CI 6.0-19.7), coughing (OR=3.3, 95% CI 1.8-5.8), fever (OR=3.2, 95% CI 1.8-5.7) or sore throat (OR=3.2, 95% CI 1.8-5.6) compared to nasal congestion. Similarly, the odds of positivity were greatest for participants from Sindh (OR=6.4, 95% CI 2.3-18.0), Baluchistan (OR=6.4, 95% CI2.3-18.0), Azad Jammu and Kashmir (OR=4.8, 95% CI, 1.3-16.9), or Federal Capital regions (OR=3.6, 95%, CI 1.4-9.6) compared to Punjab. We are conducting Sanger sequencing of the human and poultry ADV strains. Among the 28 human swabs studied thus far we found human ADV-1 (64.3%), ADV-C5 (17.9%), ADV-C89 (7.1%), ADV-B7 (7.1%), and a bovine adenovirus type 2 (3.6%). Among the 5 poultry swabs studied thus far we found only FADV-4 (22.7%). As far as we

know this is the first time a bovine adenovirus has been detected in a specimen from a sick human. These data illustrate the value of periodically conducting epidemiological studies for novel adenoviruses

GSP 46

The Search for Anti-Fungal Compounds Produced by Myxococcus xanthus

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Myxococcus xanthus, a predatory bacterium, preys upon various microorganisms, including fungi in its natural environment. Cryptococcus neoformans, a pathogenic fungus, is particularly notorious for infecting immunocompromised individuals, primarily those diagnosed with HIV/AIDS. The onset of infection typically begins in the lungs, subsequently progressing to the central nervous system, resulting in cryptococcosis and in severe cases results in cryptococcal meningitis. Annually, C. neoformans is responsible for approximately 1 million infections and 625,000 deaths. It is known that M. xanthus and other myxobacteria produce biologically active secondary metabolites that they employ to target and eliminate microorganisms in their environment. Understanding the predatory mechanism of M. xanthus towards C. neoformans holds promise for discovering novel antifungal therapies. Additionally, finding the specific genes that are responsible for the killing ability of M. xanthus is critical in developing the next generation of antifungal agents that can be effective against C. neoformans and other fungal pathogens. This study strives to elucidate potential mechanisms through which M. xanthus targets C. neoformans and assess twenty-four biosynthetic gene clusters within the M. xanthus genome to uncover secondary metabolites with antifungal properties.

GSP 47

Study the immune mechanisms of an insect-based Chikungunya chimera vaccine candidate -induced immune responses and safety in mice and guinea pigs

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Chikungunya virus (CHIKV) causes acute chikungunya fever, often accompanied by severe and persistent arthralgia. Development of safe and effective vaccines against CHIKV remains a high priority. EILV/CHIKV that contains nonstructural proteins of Eilat virus (EILV), a mosquito-specific alphavirus, and the structural proteins of CHIKV, has previously shown to protect against wild-type (WT) CHIKV challenge. The mechanism of the vaccine-induced protection remains unknown. γδ T cells, known to react to WT CHIKV infection by controlling CHIKV inflammation and tissue damage, expanded quickly in response to EILV/CHIKV vaccination. TCRδ$^{-/-}$ mice, which are deficient of γδ T cells, displayed lower levels of innate immune cytokines at day 2 post vaccination (pv). TCRδ$^{-/-}$ mice had impaired CHIKV-specific CD8^{+} T cell responses at day 28 pv and day 7 post WT CHIKV challenge. Compared to vaccinated WT group, TCRδ$^{-/-}$ mice had reduced CHIKV-specific IgG responses 28 days p.v. Type I IFNR$^{-/-}$ mice transferred with sera of vaccinated TCRδ$^{-/-}$ mice displayed more weight loss and succumbed to lethal WT CHIKV challenge more quickly compared to those treated with vaccinated WT mice sera. A sensitization study performed in guinea pigs, which were exposed to female *Ae. Albopictus* mosquitoes three times in a 2-week interval, demonstrated that EILV/CHIKV did not induce hypersensitive reactions in guinea pigs. Overall, our results suggest EILV/CHIKV is a safe vaccine candidate and induces γδ T cells -mediated protective adaptive immunity against CHIKV infection in animal models.

GSP 48

Defining the cellular and functional heterogeneity of schistosomes' esophageal gland and associated tissues.

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Schistosome flatworms cause schistosomiasis, a major parasitic disease. The esophageal gland (EG), a digestive organ, allows them to neutralize immune components while feeding on blood. The EG's development/maintenance is regulated by the transcription factor FoxA, and *foxA* RNAi leads to EG loss. This renders the parasites incapable of surviving in hosts, implicating the EG in immune evasion. Preliminary *foxA* RNAi RNA-sequencing confirms downregulation of EG genes, however; we lack a comprehensive understanding of EG cell types and genes. In closely related planarians, FoxA regulates development of the pharynx, which is composed of multiple different cell types. Additionally, existing schistosome single-cell (sc)RNA-seq and preliminary foxA RNAi RNA-seq data reveals

potential functional and cell-type diversity of EG genes, leading us to hypothesize that the EG is heterogeneous in its cell types and genes. To define EG cell types and genes, we performed head-enriched (HE) RNA-seq and scRNA-seq, enriching for EG tissue. HE scRNAseq data identified a *foxA*$^{+}$ stem cell population and two EG cell clusters. HE *foxA* RNAi RNA-seq revealed additional downregulated genes with expression patterns in and around the EG when viewed using whole-mount *in situ* hybridization, supporting the notion of EG gene heterogeneity. Additionally, pilot RNAi screens show some genes could play a role in stem cell proliferation/differentiation, which may be important for tissue maintenance and/or parasite survival. Comprehensive characterization of EG cell types and genes may identify factors that are vital for tissue homeostasis/function, with potential to be targeted for treatment of this disease.

POST DOC POSTERS:

PDP 1

Metagenomic Mining: Evolution of 'Single-Gene Lysis' Systems in Small Phages

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Bacteriophages are prokaryotic viruses that can infect, multiply, and kill the bacteria. Recent years have seen a growing interest in the discovery of phages, particularly dsDNA phages (*Caudoviricetes*), due to their potential applications in phage therapy. On the other hand, small phages like ssDNA (phiX174) and ssRNA phages (MS2,Qβ) are not as often found, even though they are studied in detail at the genomic level and used as molecular tools. Nonetheless, all these phages lyse the host bacterium. In contrast to dsDNA phages, which use a multi-gene lysis (MGL) system, ssRNA phages use a simplified lysis mechanism known as single-gene lysis (SGL). We recognize Sgl's for their ability to inhibit the peptidoglycan biosynthesis pathway, inducing autolysis. However, there are only up to 10 identified culturable ssRNA phages. Recent environmental meta-transcriptomic studies have revealed several levi-like phage genomes (*Leviviricetes*), predominantly comprising one to five genes. Typically, ssRNA phages contain four genes, with sgl being one of them. Previously, we identified many functional Sgls in meta-transcriptomic sequences that could inhibit *E. coli*. To enhance our understanding, we further evaluated the lytic activity of these Sgls against *P. aeruginosa*. According to our previous microscopic studies, Sgls cause bleb formation prior to lysis. Based on the location of blebs, Sgl activity is distinguishable as type I and II. Here, we used a methodology to categorize Sgls into operational categories based on physiological studies. The goal is to identify a novel target for inducing bacterial death as well as the potential for new antibiotic development.

PDP 2

Oxford Nanopore Technology long-read sequencing enables antimicrobial resistance predictions for emerging mechanisms of extended-spectrum beta-lactamase-producing E. coli

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Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae are resistant to common antibiotics, require complex treatments, and are classified as a serious health threat by the CDC. Importantly, *Escherichia coli* ST131 isolates carrying ESBLs of the CTX-M family spread quickly among bacteria via plasmid-mediated gene transfer. Rapid and comprehensive methods capable of differentiating emerging antimicrobial resistance mechanisms are critical for providing effective patient care and curbing increased resistance. We compared Illumina short-read and Oxford Nanopore Technology (ONT) long-read whole genome sequencing (WGS) to differentiate beta-lactam resistance in two *E. coil* strains isolated from the same patient: one urine isolate (UTI) and one blood isolate (bacteremia) following beta-lactam administration. Antimicrobial susceptibility testing showed the urine isolate was susceptible to cefepime, but the blood isolate was resistant, a finding confirmed by microbroth dilution. However, cefepime exposure did not have bactericidal effects *in vitro* for either isolate. Illumina WGS showed the two *E. coli* genomes were ST131 and 99.63% similar by wgMLST but failed to detect any differences in antimicrobial resistance gene profiles. ONT WGS confirmed the two genomes were identical (99.78%) except for an ~11 kb tandem duplication of a plasmid region containing the blaCTX-M-27 gene flanked by IS26 transposase sequences. Phenotypic characterization by targeted liquid chromatography-tandem mass spectrometry showed an increased rate

of cefepime degradation by the blood isolate over time compared to the urine isolate, which directly correlates with the CTX-M-27 gene copy number. IS26-mediated gene duplication of ESBLs is an emerging resistance mechanism that can only be discerned by long-read sequencing technology.

PDP 3

Virus-Induced Purinergic Signaling Amplifies Type 1 & III Interferon Production and Interferon Resposnes

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Rotavirus (RV) is an enteric pathogen that causes ~150,000 deaths annually in children under 5-years due to severe gastroenteritis. Innate immune responses by enterocytes are the first line of defense for establishing an antiviral 'firewall', but RV has evolved to subvert these antiviral pathways. A hallmark of RV infection is an increase in Ca^{2+} signaling both in infected and surrounding uninfected cells. Ca^{2+} signaling regulates critical innate immune pathways, but how RV-induced dysregulation of Ca^{2+} affects these responses remains uncharacterized. Recently, we found that RV-infected cells generate intercellular Ca^{2+} waves (ICWs) through the release of ADP from infected cells and paracrine activation of P2Y1 purinergic receptors on surrounding cells. ICWs are important for RV pathogenesis; however, extracellular purines are also Damage-associated Molecular Pattern (DAMP) molecules that regulate innate immune responses. Thus, these P2Y1-mediated ICWs may influence virus-induced activation of interferon (IFN) or downstream IFN-stimulated gene (ISG) responses. To test this, we examined how activation or inhibition of P2Y1 affects IFN responses stimulated by PolyI:C. Treatment with P2Y1 agonists increased both Type I and III IFN gene expression 2.5-fold, whereas P2Y1 antagonists decreased IFN responses by 1.5-fold. IFN induction was further assessed using an IFN-responsive reporter cell line. Reporter activation by IFN was significantly suppressed by P2Y1 receptor antagonists. Finally using human intestinal organoids (HIOs), we found that IFN responses were amplified by activation of P2Y1 and suppressed by P2Y1 inhibitors. These data uncover a new mechanism of innate immune signaling crosstalk between canonical virus-induced IFN and virus-triggered purinergic signaling.

PDP 4

Novel Rodent Coronavirus Detected in Beef Cattle, Mexico

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We are conducting prospective, One Health-oriented surveillance for coronaviruses (CoVs) on livestock farms in the United States and Mexico. In February 2024, we collected nasal swab samples from cattle (~70% with signs of respiratory illness) and 3-hr aerosol samples on multiple beef cattle farms near Monterrey Mexico. The samples were studied with molecular assays for CoVs, influenza A, and influenza D viruses. None of the specimens had molecular evidence of influenza A. Seventeen (42.5%) of 40 cattle nasal swabs and five (41.6%) of 12 bioaerosol samples had molecular evidence of influenza D virus. Our pan-species CoV RT-PCR assay detected CoV in 9 (22.5%) of 40 nasal swabs and in 1 (8.3%) of 12 bioaerosol samples. Sanger sequencing identified bovine CoV (n=4) and a rodent CoV (n=5) in the 9 specimens. All 5 cows with rodent CoV were sick. Influenza D virus was isolated in ST cells in 4 specimens. Attempts to isolate the rodent CoV in Vero-E6, LLC-MK2 and MDBK cells were not successful. However, using next-generation sequencing, we assembled ~33% of the rodent CoV genome from the six original samples. The virus clustered with rat CoV strains identified in China in 2011-2013. As best we know, this is the first detection of a rodent CoV in sick cattle. While we cannot rule out feed contamination, as CoV spillovers to new species are rather common, our findings are concerning. These data demonstrated the value of conducting surveillance for novel respiratory viruses on livestock farms.

FACULTY AND OTHER POSTER

FP 1

A One-Health Approach in Surveilling for Emerging Respiratory Viruses on Cattle Farms in Kentucky and Indiana

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In a 5-year prospective study, we seek to develop cost-efficient methods for detecting novel respiratory viruses on livestock farms.

Farms are visited upon enrollment and 4-, 8-, and 12-month intervals. Between visits, farms can ship specimens from sick livestock or workers. Specimens are examined with molecular assays for influenza A, influenza D, and coronaviruses with unique virus detections further studied with next-generation sequencing (NGS).

Six Kentucky and Indiana farms yielded 327 samples in 2024: 168 cattle nasal, 2 cattle ocular, 3 cow lung necropsy, 3 dead-bird, 37 human nasopharyngeal, 93 bioaerosol, and 21 environmental. No Influenza A was detected. 11 (6.5%) of 168 cattle nasal swabs and 2 water pen samples had evidence of influenza D. 53 (31.5%) of 168 cattle nasal swabs and 2 ocular swabs had evidence of coronavirus. Eighteen (34%) of these 53 cattle tested had respiratory signs. Sanger sequences from 29 (54.7%) of the 53 had evidence of bovine coronavirus. NGS analysis of the hemagglutinin-esterase precursor (HEF) gene in influenza D viral strains showed phylogenetic similarity. We also detected/assembled 7 additional viruses from cattle nasal swabs: bovine rhinitis A (100% genome coverage), bovine coronavirus (99.9%), bovine nidovirus (98.4%), enterovirus E (97.3%), Praha dicistro-like virus 2 (90.9%), bovine rotavirus (88.9%), bovine rhinitis B (82.9%), and flumine dicistrovirus 40 (72.7%). Praha dicistro-like virus 2 and Flumine dicistrovirus 40 have not been previously detected in cattle.

These data demonstrate the potential of a One Health-approach in surveilling for novel respiratory viruses on livestock farms.

FP 2

A New Phylogenetic and Sequence Analysis of Penicillin-Binding Proteins

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Penicillin-binding proteins (PBPs) are transpeptidases that are essential in forming cell walls in many bacteria. These proteins are the targets of *beta*-lactam antibiotics, including the penicillins, cephalosporins, carbapenems, and monobactams. These proteins are of interest due to the increasing levels of bacterial resistance to antibiotics, including to the *beta*-lactams.

Overcoming resistance to *beta*-lactam antibiotics has mainly involved studies on the *beta*-lactamases, bacterial enzymes of various types that open, and thus inactivate, the antiobiotic *beta*-lactam ring. One other way that can be used to study bacterial resistance to antibiotics is to study the antibiotic's target, the PBP's, in order to discover new, non-lactam compounds that might replace the current generation of antibiotics.