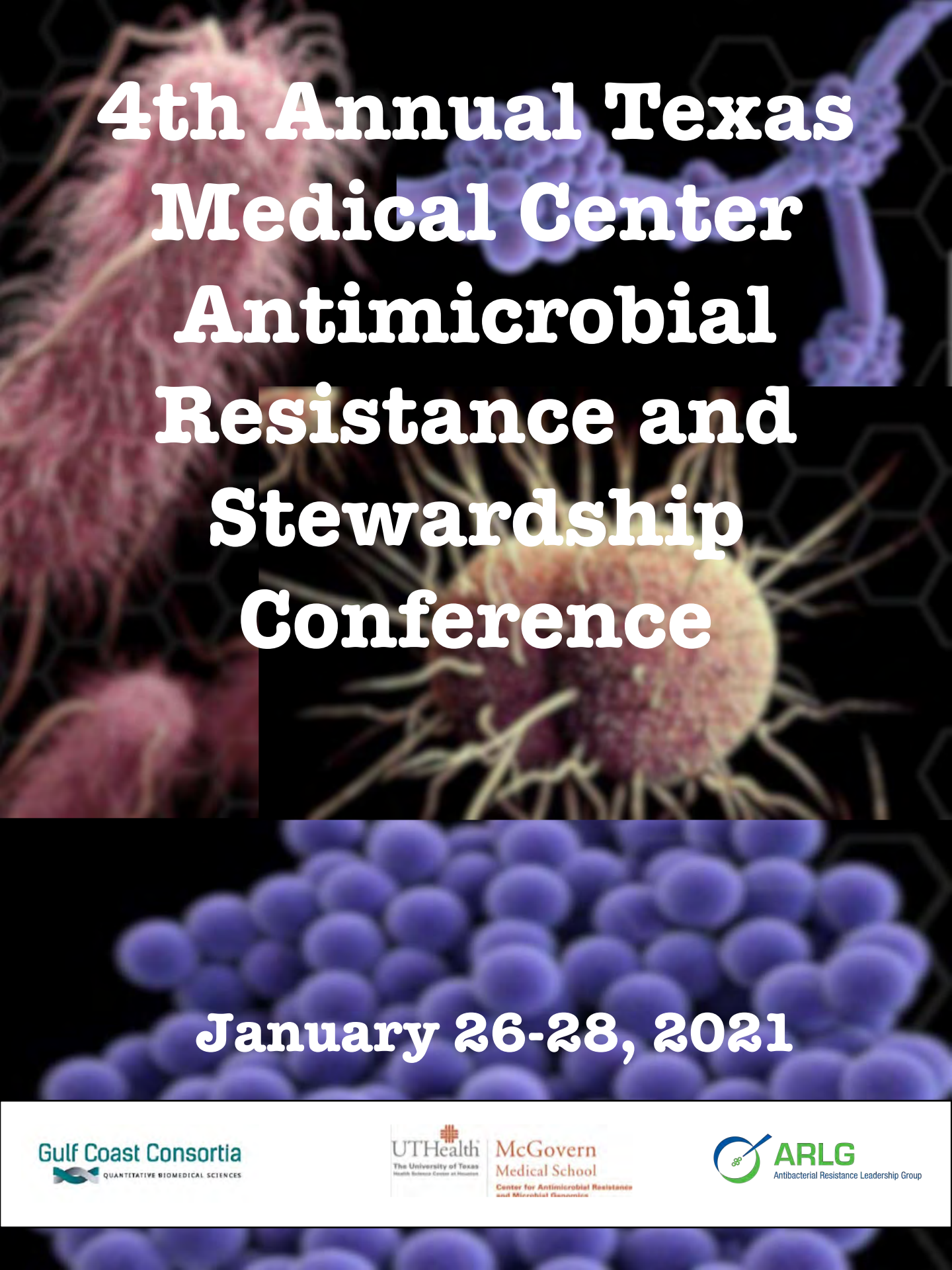

4th Annual Texas Medical Center Antimicrobial Resistance and Stewardship Conference

January 26-28, 2021

Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

UTHealth
The University of Texas Health Science Center at Houston
McGovern Medical School
Center for Antimicrobial Resistance and Microbial Genomics

ARLG
Antibacterial Resistance Leadership Group

Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi- institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting-edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Pharmacological Sciences, Precision Environmental Health Sciences and Antimicrobial Resistance. GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include AI in Healthcare, Antimicrobial Resistance, Cellular and Molecular Biophysics, Innovative Drug Discovery and Development, Immunology, Mental Health, Regenerative Medicine, Single Cell Omics, Theoretical and Computational Neuroscience, Translational Imaging and Translational Pain Research. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.

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Day 1 - Tuesday, January 26, 2021

Mechanisms of Resistance and Drug Discovery

7:30-8:30 *Career Mentoring: Women in Infectious Disease Research*
Danielle Garsin, PhD
Professor, University of Texas Health Science Center, Houston
Carol Baker, MD
Professor**,** University of Texas Health Science Center, Houston
Sara Cosgrove, MD
Professor, John Hopkins, Baltimore

8:30-8:35 *Welcome*
Cesar A. Arias, MD, PhD
Professor, University of Texas Health Science Center, Houston

8:35-9:00 *Clinical Research on Antibiotic Resistance*: *Antimicrobial Resistance Leadership Group*
Henry Chambers, MD
Professor, University of California, San Francisco

Session 1

Conveners: **Cecilia Tran**, PharmD, Assistant Professor, University of Texas Health Science Center, Houston
Ayesha Khan, PhD, Post-doctoral Research Fellow, University of Texas Health Science Center, Houston

9:00-9:25 *Novel Insights into the Mechanism of Daptomycin Action*
Hans Georg Sahl, PhD
Emeritus Professor, University of Bonn, Germany

9:25-9:50 *Specific Host-pathogen Interactions of Enterococcus Faecalis*
William Miller, MD
Assistant Professor, University of Texas Health Science Center, Houston

9:50 -10:15 *Heteroresistance in Gram-negative Bacteria*
David Weiss, **PhD**
Associate Professor, Emory University, Atlanta

10:15-10:45 **Vendor Show and Networking**

Session 2 *T32 Trainee Symposium: Texas Medical Center Training Program on Antimicrobial Resistance (TP-AMR), Emory Training Program on Antimicrobial Resistance, University of Pittsburgh Training Program on Antimicrobial Resistance*

Conveners: **Dierdre Axell-House**, MD, Infectious Disease Fellow, Baylor College of Medicine, Houston
James Budnick, PhD, Postdoctoral Scholar, University of Pittsburgh

10:45-11:00 *Experimental Evolution of Acinetobacter baumannii during Combinational Antibiotic Exposure*
Francine Arroyo, PhD
Postdoctoral Research, University of Pittsburgh

11:00-11:15 *Host-directed Therapeutics for the Treatment of Bacterial Infections*
Jourdan Andersson, PhD
Industry

11:15-11:30 *Characterization of a LysR Regulator in MDR A. baumannii*
Aimee Tierny
PhD Graduate Student, Emory University, Atlanta

11:30-11:45 *Contribution of a Mobile Genetic Element to Adherence, Invasion and Pathogenesis of Emergent Antimicrobial Resistant Group A Streptococcus*
Luis Vega, PhD
Post-doctoral Research Fellow, University of Texas Health Science Center, Houston

11:45 -12:15 **Key Note Lecture**
Discovering New Antibiotics: State of the Art
Gerry Wright, **PhD**
Professor, University of McMaster, Hamilton, Canada

12:15-12:30 **Break**

12:30-1:05 **Rapid Fire Presentations**

Convener: **Eva Preisner, PhD**
Postdoctoral Researcher, Baylor College of Medicine, Houston

Pyoverdine Antivirulents Synergize with Gallium Nitrate to Inhibit Pseudomonas aeruginosa
Alex Deyanov, Graduate Student, Rice University
Poster 19

Cellular Response of C. difficile Epidemic 027 to Metronidazole-induced Oxidative Stress
Abiola Olaitan, Postdoc, IBT, Texas A&M
Poster 20

Role of the LiaF in the LiaR-Mediated Response Against Daptomycin and Antimicrobial Peptides in Multidrug-Resistant Enterococcus faecalis (Efs)
Diana Panesso, Assistant Professor, UT Health Science Center Houston
Poster 21

Using Complex Microbial Communities to Identify Microbes with Cryptic Antibiotic Potential
Paul "Skip" Price, Assistant Professor, Eastern Michigan Univ.
Poster 22

The MacAB Efflux Pump is Involved in Protecting Serratia marcescens from Aminoglycoside Antibiotics, but not from Macrolide Antibiotics
CeciliaSierra-Bakhshi, Graduate Student, Marshall University
Poster 23

Deciphering the Determinants of KPC-2 Carbapenemase Activity and Substrate Specificity Using Random Mutagenesis and Deep Sequencing
Zhizeng Sun, Assistant Professor, Baylor College of Medicine
Poster 24

Structural and Biochemical Studies of MurAA, an Enolpyruvate Transferase that Contributes to Cellular Fitness During Daptomycin Attack in Enterococcus faecium
Yue Zhou, Graduate Student, Rice University
Poster 25

1:05-1:45 **Poster Session, Posters 1-25**

1:45-2:00 **Break**

Session 3

Conveners: **Lynn Zechiedrich**, PhD, Professor, Baylor College of Medicine, Houston
Julian Hurdle, PhD, Associate Professor, Institute of Biotechnology, Texas A&M University, Houston

2:00-2:25 *Multi-Dimensional Antibacterialsut*
Anthony Maresso, PhD
Associate Professor, Baylor College of Medicine, Houston

2:25-2:50 *Novel Peptides with Antifungal Activity*
Danielle Garsin, PhD
Professor, University of Texas Health Science Center, Houston

2:50-3:15 *Novel β-lactam/β-lactamase Inhibitors*
Robert Bonomo, MD
Professor, Case Western Reserve University, Cleveland

3:15-3:30 **Break**

Session 4

Conveners: **Anthony Harris** and **Melinda Pettigrew**
NIH Antimicrobial Resistance Leadership Group (ARLG)

3:30-3:45 *ARLG's Role in Supporting Future Generations of ID Investigators*
Anthony Harris, MD
Professor, University of Maryland, Baltimore
Melinda Pettigrew, MD
Professor and Senior Associate Dean of Academic Affairs, Yale School of Public Health

3:45-4:00 *Early Stage Investigators Seed Grants*
Ritu Banerjee, MD, PhD
Associate Professor, Vanderbilt University, Nashville

4:00-4:15 *ARLG Fellowship*
Judith Anesi, MD
Assistant Professor, University of Pennsylvania

4:15-4:30 *Trialist in Training*
Michael Satlin, MD
Associate Professor, Weil Cornell Medical College, New York

4:30-4:45 Wrap-Up
Anthony Harris, MD
Professor, University of Maryland, Baltimore

Session 5

Convener: **Ashok Chopra**, PhD**,** Professor, University of Texas Medical Branch, Galveston

4:45-5:00 *Detection of CTX-M-27 β-Lactamases on Two Distinct Plasmid Types in ST38 Escherichia coli from Several US States*,
Andrew Cameron, PhD
Postdoc, University of Rochester

5:00-5:15 *An Outbreak of Candida auris During a COVID-19 Case Surge in Florida*
Bhavarth Shukla, MD
Assistant Professor, University of Miami

5:15-5:30 *Phenotypic and Genotypic Characterization of Viable Bacteria from Cattle Feedyard Dust*
Maribel Leon
Graduate Student, Texas A&M Univeristy

Day 2 - Wednesday, January 27, 2021

Translational and Clinical Aspects of Antibiotic Resistance

7:30-8:30 *Career Mentoring: Career Development Awards in Antimicrobial Resistance*
Cecilia Tran, PharmD
Assistant Professor, University of Texas Health Science Center, Houston
William Miller, MD
Assistant Professor, University of Texas Health Science Center, Houston
Blake Hanson, PhD
Assistant Professor, University of Texas Health Science Center, School of Public Health, Houston

Moderators: **Cesar Arias**, MD, PhD
Professor, University of Texas Health Science Center, Houston
William Shafer, PhD
Professor, Emory University, Atlanta

Session 6

Conveners: **Tim Palzkill**, PhD
Professor, Baylor College of Medicine
Charlene Offiong, PharmD
Houston Health Department

8:30-8:55 *Detecting ESBL-carrying Gram-negative Bacteria: the Aftermath of MERINO*
Pranita Tamma, MD
Associate Professor, Johns Hopkins University, Baltimore

8:55-9:20 *Genomics of Carbapenem-resistant Enterobacterales in the USA*
Blake Hanson, PhD
Assistant Professor, University of Texas Health Science Center, School of Public Health, Houston

9:20-9:45 *Whole Genome Sequencing and Detection of Resistance in the Routine Clinical Laboratory*
Robin Patel, MD
Infectious Disease Specialist, Mayo Clinic, Rochester

9:45-10:15 **Vendor Show and Networking**

Session 7 *T32 Trainee Symposium: Texas Medical Center Training Program on Antimicrobial Resistance (TP-AMR), Emory Training Program on Antimicrobial Resistance, University of Pittsburgh Training Program on Antimicrobial Resistance*

Conveners: **Luis Vega, PhD**
Postdoctoral Research Fellow, University of Texas Health Science Center, Houston
Aimee Tierny, PhD
Graduate Student, Emory University, Atlanta

10:15-10:30 *LiaX as a Surrogate Marker of Daptomycin Susceptibility in Multidrug-Resistant Enterococcus faecium Recovered from Cancer Patients*
Dierdre Axell-House, MD
Infectious Diseases Fellow, Baylor College of Medicine, Houston

10:30-10:45 *Mechanism of 50S Ribosomal Subunit Recognition and Modification by the Mycobacterium tuberculosis rRNA Methyltransferase TlyA*
Zane Laughlin
Graduate Student, Emory University, Atlanta

10:45-11:00 *Identifying Mechanisms of Intrinsic Antimicrobial Resistance in Klebsiella pneumoniae by in vitro Evolution*
James Budnick, PhD
Postdoctoral Scholar, University of Pittsburgh

11:00-11:15 *Simplified Microbial Communities as a Safe Antimicrobial Treatment Option in Clostridioides difficile infections*
Eva Preisner, PhD
Postdoctoral Researcher, Baylor College of Medicine, Houston

11:15-11:30 *Whole Genome Sequencing Surveillance for Prevention of Antibiotic-Resistant Infections*
Lee Harrison, **MD**
Professor, University of Pittsburgh

11:30-12:15 **Key Note Lecture**
Clinical Trials on Antibiotic Resistance
Vance Fowler, MD
Professor of Medicine, Duke University, Durham

12:15-12:30 **Break**

Convener: **April Nguyen**, Graduate Student, Univeristy of Texas Health Science Center Houston

12:30-1:05 **Rapid Fire Presentations**

Predictive Regulatory and Metabolic Network Models for Systems Analysis of Clostridioides difficile
Mario Arrieta-Ortiz, Postdoc, Institute for Systems Biology
Poster 43

Amino Acid Substitutions in Key Regions of BlaI and BlaR Correlate with the Cefazolin Inoculum Effect in Methicillin Susceptible Staphylococcus aureus (MSSA)
Sara Gomez-Villegas, Postdoc, UT Health Science Center Houston
Poster 44

Chemical Genetic Exploration of Clostridium difficile Toxin Metabolism, Toward Defining Anti-virulent Drug Targets
RaviKanthReddy Marreddy, Postdoc, Texas A&M IBT
Poster 45

Target Validation of Novel Antibiotic, Peptide-Conjugated Phosphorodiamidate Morpholino Oligomers (PPMOs)
Amila Nanayakkara, Postdoc, UT Southwestern
Poster 46

Discovery of Inhibitors of the KPC-2 Carbapenemase Using a Focused DNA-Encoded Library
Jane Park, Graduate Student, Baylor College of Medicine
Poster 47

Anti-Viral Resistance and Phage Counter Adaptation to Pandemic E. coli
Keiko Salazar, Graduate Student, Baylor College of Medicine
Poster 48

Annotation-Agnostic Metagenomic Biomarkers of Infectious Disease Susceptibility
Charlie Seto, Postdoc, Baylor College of Medicine
Poster 49

1:05-1:45 **Poster Session Posters 26-49, 62**

1:45-2:00 **Break**

Session 8

Conveners: **German Esparza, MSc**
Professor, Universidad Javeriana, Bogota, Colombia

2:00-3:00 *Challenging Clinical Cases on Antimicrobial Resistance: Susceptibility Interpretation*
Jose Munita, MD
Associate Professor, Universidad del Desarrollo, Santiago, Chile
Helen Boucher, MD
Professor of Medicine, Tufts University, Boston
Robin Patel, MD
Infectious Diseases Specialist, Mayo Clinic, Rochester

Session 9

Conveners: **Henry Chambers, MD**
Professor, University of California, San Francisco
Vance Fowler, MD
Professor of Medicine, Duke University, Durham

Research Focus Areas for the Antimicrobial Resistance Leadership Group

3:00-3:15 *DOTS Study*
Tom Holland, MD
Associate Professor, Duke University, Durham

3:15-3:30 *RADICAL Study*
Ephraim Tsalik, MD, PhD
Associate Professor, Duke University, Durham

3:30-3:45 *Diagnostics MASTER-GC*
Sarah Doernberg, MD
Associate Professor, University of California, San Francisco

3:45-4:00 *"All of Us are Better Than Any of Us": Early Insights in Global Clinical and Molecular Epidemiology of Carbapenem-Resistant Enterobacterales*
Minggui Wang, MD
Professor of Medicine**,** Huashan Hospital, Fudan University, China

4:00-4:30 **Vendor Show and Networking**

Session 10: Microbiome Session

Conveners: **Herbert Dupont, MD**
Professor, University of Texas Health Science Center, School of Public Health, Houston
Charles Darkoh, PhD
Associate Professor, University of Texas Health Science Center, School of Public Health, Houston

4:30-4:55 *Microbiome, Bacteria and Cancer*
Robert Jenq, MD
Associate Professor, University of Texas MD Anderson Cancer Center, Houston

4:55-5:20 *Microbiome, Nutrients and Clostridioides Difficile*
Robert Britton, PhD
Professor, Baylor College of Medicine

5:20-5:45 *Microbiome Transplantation in Chronic Diseases*
Netanya Utay, MD
Associate Professor, University of Texas Health Science Center, Houston

Day 3 – Thursday, January 28, 2021

Antibiotic Stewardship

7:30-8:30 *Career Mentoring: Meet the Professors, Clinical Scientist Track, MDs and PharmDs*
Ed Septimus, MD
Professor, Harvard University and Texas A&M College of Medicine, Houston
Trish Perl, MD
Professor, University of Texas Southwestern, Dallas
Vincent Tam, PharmD
Professor, University of Houston, Houston

Session 11

Convener: **Ed Septimus, MD**
Professor, Harvard University and Texas A&M College of Medicine, Houston

8:30-8:35 Welcome
Ed Septimus, MD
Professor, Harvard University and Texas A&M College of Medicine, Houston

8:35-9:15 **Keynote Lecture**
The Role of HAI Prevention and Antimicrobial Resistance
Trish Perl, MD
Professor, University of Texas Southwestern, Dallas

9:15-9:45 *Pediatrics Surviving Sepsis Campaign*
Andrea Cruz, MD
Associate Professor, Baylor College of Medicine, Houston

9:45-10:15 *Refocusing Antimicrobial Stewardship Efforts to Optimize Patient Outcomes*
Jason Pogue, PharmD
Clinical Pharmacy Specialist, University of Michigan

10:15-10:45 **Vendor show and networking**

Session 12

Conveners: **Kristi Kuper**, PharmD
Tabula Rasa/DoseMe Rx

10:45-11:15 *The Veteran Health System Experience*
Matt Goetz, MD
Chief Infectious Diseases, University of California Veterans Affairs, Los Angeles

11:15-11:45 *Hospitalists Role in Antimicrobial Stewardship*
Scott Flanders, MD
Professor, University of Michigan

11:45-12:15 Panel: *Clinical Cases*
Ed Septimus, MD
Professor, Harvard University and Texas A&M College of Medicine, Houston
Daniel Musher, MD
Distinguished Service Professor, Baylor College of Medicine, Houston
Vance Fowler, MD
Professor of Medicine, Duke University, Durham

Convener: **Ayesha Khan**, PhD, Post-doctoral Research Fellow, University of Texas Health Science Center, Houston

12:15-12:45 Rapid Fire Presentations

Urine Culture High Contamination Rates call into Question the Gold Standard for Urinary Tract Infections
Michael Hansen, Assistant Professor, Baylor College of Medicine
Poster 68

Back to The Future: Increasing Penicillin Susceptibility among Methicillin-Susceptible Staphylococcus aureus Osteoarticular Infections in Children
Jonathon McNeil, Assistant Professor, Baylor College of Medicine
Poster 69

Development of Bacteriophages with Anti-Biofilm Properties as Novel Treatment for Catheter-Associated Urinary Tract Infections
BelkysSánchez, Postdoc, Baylor College of Medicine
Poster 70

Non-carbapenemase Producing Organisms with CTX-M Gene Amplification Account for Majority of Invasive Carbapenem-resistant Enterobacterales bacteremia in Immunocompromised Patient Population
William Shropshire, Graduate Research Assistant, UT Health Science Center Houston
Poster 72

Characterization of the Antimicrobial Susceptibility Patterns and Virulence Mechanisms Promoting Staphylococcal Medical Device Infections
Jennifer Walker, Assistant Professor, UT Health Science Center Houston
Poster 73

12:40-12:45 Break

12:45-1:25 **Poster Session Posters 50-61, 63-76**

1:25-1:30 Break

1:30-2:00 Panel Discussion: *The Houston Response to COVID-19 Lessons Learned*
Umair Shah, MD
Secretary of Health, Washington State
David Persse, MD
Physician Director, City of Houston

Session 13

Convener: **Kevin Garey, PharmD**
Professor, University of Houston

2:00-2:30 *Update on Rapid Diagnostics*
Katherine Perez, PharmD
Assistant Professor, Houston Methodist Hospital, Houston

2:30-3:00 *HIV Treatment and Resistance*
Tom Giordano, MD
Professor & Section Chief, Baylor College of Medicine, Houston

3:00-3:30 Panel Discussion: *Immunization*
Carol Baker, MD
Professor, University of Texas Health Science Center, Houston
Flor Muñoz, MD
Associate Professor, Baylor College of Medicine/Texas Children's Hospital, Houston
Robert Atmar, MD
Professor, Baylor College of Medicine, Houston

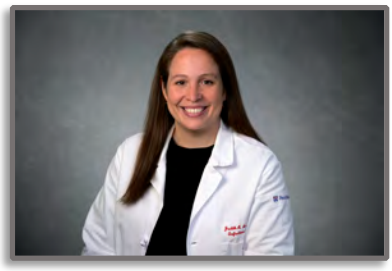
3:30-3:45 *Closing Remarks*
Cesar A. Arias, MD, PhD
Professor, University of Texas Health Science Center, Houston

Ed Septimus, MD
Professor, Harvard University and Texas A&M College of Medicine, Houston

Speakers in Alphabetical order

Jourdan Andersson, PhD
Research Scientist
Host-directed Therapeutics for the Treatment of Bacterial Infections

Dr. Jourdan Andersson is part of the first cohort of trainees in the Texas Medical Center Training Program in Antimicrobial Resistance (TPAMR). She is a former postdoctoral fellow in the laboratory of Dr. Tor Savidge at Baylor College of Medicine and Texas Children's Hospital in the Texas Children's Microbiome Center, Houston, TX. She received her B.S degree from Rensselaer Polytechnic Institute and Ph.D. from the University of Texas Medical Branch. Her research during her fellowship focused on identifying alternative host-mediated therapeutics to combat antibiotic resistant pathogens, specifically Clostridioides difficile and Klebsiella pneumoniae.



Judith A. Anesi, MD, MSCE
Assistant Professor
Epidemiology
ARLG Fellowship

Dr. Anesi is an Assistant Professor of Medicine and Epidemiology in the Division of Infectious Diseases at the University of Pennsylvania. Her research focuses on the clinical and molecular epidemiology of bacterial infections among solid organ transplant recipients, with a unique expertise in donor-derived bacterial infections, multidrug-resistant organisms (MDROs), and healthcare-associated infections. She is the recipient of a five-year NIAID Career Development Award (K01), for which she is performing a prospective multicenter study that aims to determine the incidence of, risk factors for, and impact of DDBIs on transplant recipient outcomes, with a particular focus on MDROs.

Cesar A. Arias, MD, MSc, PhD, FIDSA
Professor
Infectious Disease

Cesar A. Arias, M.D. MSc, Ph.D. is Professor and the Margaret and Herbert Dupont Chair in Infectious Diseases and holds the Laurel and Robert H. Graham Faculty Fellowship at McGovern Medical School. He is the director and founder of the Center of Antimicrobial Resistance and Microbial Genomics (CARMiG) at McGovern Medical School and the Center for Infectious Diseases at the UTHealth School of Public Health. Dr. Arias obtained his medical degree from Universidad El Bosque, Bogota, Colombia (first in class) and then spent 6 years in the United Kingdom where he obtained Masters in Clinical Microbiology at The University of London and a PhD in Microbial Biochemistry and Molecular Microbiology at University of Cambridge. He then was awarded a Wellcome Trust International Fellowship to develop antimicrobial resistance research in Colombia, where he founded the Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics at Universidad El Bosque, Bogota. In 2002, he moved to Houston for training in Internal Medicine and Infectious Diseases at UTHealth McGovern Medical School and MD Anderson Cancer Center. He joined the UTHealth faculty as assistant professor in 2008 and became professor in 2016.

Dr. Arias is a nationally and internationally recognized expert conducting NIH-funded basic, translational and clinical research on mechanisms of antibiotic resistance with emphasis on Gram-positive organisms (in particular enterococci). His expertise also includes the clinical impact of resistance and the molecular epidemiology of antibiotic-resistant organisms, using state-of-the-art genomic analyses. He was one of the first recipients of the NIH K99/R00 Pathway to Independence Award and has

also been the recipient of the American Society of Clinical Microbiology Young Investigator Award and the Infectious Diseases Society Oswald Avery Award for early achievement, among others. He has been active in professional societies including serving in the Program Planning Committee of IDWeek since 2014, serving as Vice-Chair (2018) and Chair (2019). He was inducted to the American Society for Clinical Investigation in 2015. He serves as Editor In Chief of Antimicrobial Agents and Chemotherapy and is the Chair of the Gulf Coast Consortium on Antimicrobial Resistance in Houston (a partnership between 7 institutions in the Texas Medical Center). Dr. Arias carries out projects with a network of collaborators across the Texas Medical Center and international sites using multidisciplinary approaches.

Francine Arroyo, PhD
Postdoctoral Fellow

Experimental Evolution of Acinetobacter baumannii During Combinational Antibiotic Exposure

Dr. Arroyo is widely interested in how bacterial lifestyle affects adaptations to abiotic and biotic stresses in the environment. She studies “extreme” bacterial examples of adaptation to better understand the limits of microbial life in these systems. She earned a M.S. in Biology at Humboldt State University studying iron and sulfur oxidizing bacteria from hot acidic environments under the mentorship of Dr. Patricia Siering. She was awarded a PhD in Microbiology from Cornell University in the lab of Prof. Esther Angert. Her doctoral thesis used bioinformatics to study the ecology and evolution of giant gut microbes (Epulopiscium sp. and relatives), found in tropical surgeonfish. She joined Prof. Vaughn Cooper’s lab to investigate the role of biofilm formation on the evolution of antimicrobial resistance in the multidrug resistant pathogen Acinetobacter baumannii. She is an NIH-funded T32 Postdoctoral Fellow from the division of Infectious Diseases.

Robert L. Atmar, MD
Professor
Infectious Diseases
Panel Discussion*: Immunization*

Robert L. Atmar, M.D., is the John S. Dunn Research Foundation Clinical Professor in Infectious Diseases in the Departments of Medicine and Molecular Virology & Microbiology at Baylor College of Medicine. He received a Bachelors of Science degree in Biology from Texas A&M University in 1978 and his medical degree from Baylor College of Medicine in 1981. He completed an internship and residency in Internal Medicine and fellowship in Infectious Diseases at Baylor College of Medicine. He is a member of BCM's Vaccine Treatment & Evaluation Unit and the Digestive Diseases Center, and he also serves as the chief of the Infectious Diseases Service at Ben Taub General Hospital. Dr. Atmar's research interests include the epidemiology, pathogenesis, diagnosis, treatment and prevention of viral respiratory and enteric infections. He has studied influenza and noroviruses for more than 25 years, with a special emphasis on the diagnosis, clinical evaluation, and immunology of these viral infection and on the evaluation of vaccine candidates and strategies to prevent disease caused by these viruses. He is a Fellow in the IDSA, ACP and SHEA, and he serves as the North American editor for the *Journal of Infection.*

Dierdre Axell-House, MD
Infectious Diseases Fellow Physician
Postdoctoral Fellow

LiaX as a Surrogate Marker of Daptomycin Susceptibility in Multidrug-Resistant Enterococcus faecium Recovered from Cancer Patients

Dierdre Axell-House, MD is an Infectious Diseases fellow physician at Baylor College of Medicine with a focus on immunocompromised patients with MD Anderson Cancer Center, and a T32 postdoctoral fellow in the Training Program in Antimicrobial Resistance (TPAMR) in the Texas Medical Center. She previously completed her residency in Internal Medicine at University of Virginia in Charlottesville, VA and medical school at Jefferson Medical College in Philadelphia, PA. Her research in the TPAMR regards developing a diagnostic test for daptomycin resistance in multidrug-resistant enterococci in the lab of Cesar A. Arias, MD PhD.

Carol J. Baker, MD
Adjunct Professor
Pediatrics
Immunization Panel Discussion

Dr. Baker is adjunct professor of pediatrics, McGovern Medical School at the University of Texas Health Science Center, Houston.

Dr. Baker's clinical research has focused on all aspects of group B streptococcal (GBS) infections from the first description of early-and late-onset sepsis and meningitis in neonates and young infants to the discovery of critical epitopes in the capsular polysaccharides necessary for development of candidate conjugate vaccine candidates. Her policy, advocacy and leadership role at the CDC's Advisory Committee on Immunization Practices led to the prevention of young infant influenza and pertussis disease through maternal immunization.

Among numerous honors Dr. Baker has received the Mentor Award (2008), Society Citation for outstanding achievements in the field of Infectious Disease (2011) and Alexander Fleming Award for Lifetime Achievement (2016), each from the Infectious Diseases Society of America (IDSA). She has the Distinguished Physician and Distinguished Research Awards from the Pediatric Infectious Diseases Society, was the 2019 recipient of the Albert Sabin Gold Medal Award from the Sabin Vaccine Institute and is an elected member of the National Academy of Science. She has published more than 400 peer reviewed papers and was Associate Editor of 5 editions of American Academy of Pediatrics Red Book.

Ritu Banerjee, MD, PhD
Associate Professor
Pediatric Infectious Diseases
Early Stage Investigators Seed Grants

Dr. Ritu Banerjee is an Associate Professor in the Division of Pediatric Infectious Diseases at Vanderbilt University Medical Center. She is the Director of the Antimicrobial Stewardship Program at Vanderbilt's Children's Hospital and the Program Director of the Pediatric Infectious Diseases fellowship at Vanderbilt. She received her MD and Ph.D degrees from Washington University in St. Louis and then completed Pediatrics residency and Pediatric Infectious Disease fellowship at the University of California, San Francisco. She is a member of the PIDS Committee on Antimicrobial Stewardship, the AAP Committee on Infectious Diseases, and leads the Pediatrics Working Group of the Antibacterial Resistance Leadership Group (ARLG) of NIAID. Dr. Banerjee conducts clinical research about antibiotic stewardship and implementation and outcomes of rapid diagnostics for infectious diseases.

Helen W. Boucher, MD, FACP, FIDSA

Chief, Division of Geographic Medicine and Infectious Diseases
Director, Levy Center for Integrated Management of Antimicrobial Resistance (CIMAR)
Director, Heart Transplant and Ventricular Assist Device Infectious Diseases Program
Professor of Medicine
Division of Geographic Medicine and Infectious Diseases

Challenging Clinical Cases on Antimicrobial Resistance: Susceptibility interpretation

Helen Boucher is the Chief of the Division of Geographic Medicine and Infectious Diseases and Director of the Stuart B.Levy Center for Integrated Management of Antimicrobial Resistance (CIMAR), a collaborative, cross-disciplinary initiative between Tufts University and Tufts Medical Center with a mission of innovating to protect humanity from the global threat of antimicrobial resistance by integrating solutions across human and veterinary medicine, stewardship and awareness. She is Director of TMC's Heart Transplant and Ventricular Assist Device Infectious Diseases Program, and Professor of Medicine at Tufts University School of Medicine.

Dr. Boucher's clinical interests include infections in immunocompromised patients and S. aureus infections. Her research interests focus on S. aureus and the development of new anti-infective agents. She is the author or coauthor of numerous abstracts, chapters, and peer-reviewed articles, which have been published in such journals as The New England Journal of Medicine, Antimicrobial Agents and Chemotherapy, Clinical Infectious Diseases, and The Annals of Internal Medicine; she is Associate Editor of Antimicrobial Agents and Chemotherapy.

In 2015, Dr. Boucher was appointed a voting member of the Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria, and elected Treasurer of the Infectious Diseases Society of America. She was awarded the IDSA Society Citation Award in October, 2015. Dr. Boucher serves on the Board of Trustees of The College of the Holy Cross and as Chair of the Board of Trustees of the Physicians of Tufts Medical Center.

Robert A. Bonomo, MD
Professor
Medicine, Pharmacology, Molecular Biology and Microbiology, Biochemistry, and Proteomics and Bioinformatics

Novel β-lactam/β-lactamase Inhibitors

Dr. Robert A. Bonomo is Distinguished University Professor of Medicine, Pharmacology, Molecular Biology and Microbiology, Biochemistry, and Proteomics and Bioinformatics at Case Western Reserve University School of Medicine. He also serves as Associate Chief of Staff for Academic Affairs at the Louis Stokes Cleveland VA Medical Center, Director of the Cleveland Geriatric Research and Education Clinical Care Center, Vice Chair for Veterans Affairs in the University Hospitals Case Medical Center Department of Medicine and Director, CWRU-Cleveland VAMC Center for Antimicrobial Resistance and Epidemiology (Case VA CARES). His research interests include the mechanistic basis of resistance to ß-lactam antibiotics and ß-lactamase inhibitors in Gram negative and Mycobacteria, the molecular epidemiology of multidrug resistant Gram-negative bacteria, infections in the elderly, and the implementation of molecular diagnostics in clinical care of patients with infectious disease. He is an elected fellow of the Infectious Diseases Society of America, American Academy of Microbiology, the Association of American Physicians, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and has been appointed to PCORI as a representative of the Infectious Diseases Society of America. He is also Co-Director of the Laboratory Center of the NIH Sponsored Antibacterial Resistance Leadership Group (ARLG).

Robert Britton, PhD
Professor
Molecular Virology and Microbiology
Microbiome, Nutrients and Clostridioides Difficile

Dr. Robert Britton is a Professor in the Department of Molecular Virology and Microbiology, a Member of the Alkek Center for Metagenomics and Microbiome Research and the Director of the Microbial Cultivation Center at Baylor College of Medicine. He currently leads the Therapeutic Microbiology laboratory that is focused on the use of microbes to prevent and treat human disease. Currently funded research projects in the Britton laboratory range from how intestinal microbial communities resist invasion by the diarrheal pathogen C. difficile to the development of bacterial biosensors for the detection and treatment of intestinal inflammation. His laboratory has made several advances in the development of genetic and microbial growth platforms to aid in the understanding of microbes that promote health and disease. These include the development of precision genome engineering technologies for lactic acid bacteria and the development of human fecal minibioreactor arrays to study the function of microbial communities in a high-throughput manner. Dr. Britton received a B.S. in Biology from the University of Nebraska-Lincoln and a Ph.D. in Cell and Molecular Biology from Baylor College of Medicine. After performing postdoctoral training at MIT he started his own laboratory at Michigan State University in 2003. After rising to the rank of Professor in 2014 he moved to his current position at Baylor College of Medicine.

James Budnick, PhD
Postdoctoral Fellow
Identifying Mechanisms of Intrinsic Antimicrobial Resistance in Klebsiella pneumoniae by in vitro Evolution

James (Jimmy) Budnick is a T32 Postdoctoral Fellow in the University of Pittsburgh School of Medicine Training Program in Antimicrobial Resistance (TPAR). His research is focused on uncovering mechanisms of intrinsic antibiotic resistance in the opportunistic pathogen Klebsiella pneumoniae. He's conducting his research in the lab of Dr. James Bina in the Department of Microbiology and Molecular Genetics at Pitt and is a recipient of the 2020 Catalyst Award from the University of Pittsburgh Center for Evolutionary Biology and Medicine.

Andrew Cameron, PhD
Departmental Fellow
Clinical Microbiology

Detection of CTX-M-27 β-Lactamases on Two Distinct Plasmid Types in ST38 Escherichia coli from Several US States

Andrew Cameron is an ASM CPEP Fellow in Clinical and Public Health Microbiology at the University of Rochester Medical Centre (Rochester, NY). In 2015, he received a Ph.D in Microbiology and Immunology from the University of British Columbia (Vancouver, BC, Canada) under the mentorship of Erin C. Gaynor, characterizing high frequency genetic variation in the gastrointestinal pathogen *Campylobacter jejuni*. Following this, he became interested in antibiotic resistance and joined the Government of Canada's Postdoctoral Research Program in the laboratory of Tim A. McAllister, where he contributed to identification of resistance mechanisms, whole-genome phylogenetic One Health surveillance of *Enterococcus* and *E. coli*, and characterization of mobile genetic elements in drug-resistant veterinary pathogens, including *Pasteurella multocida*. Currently, he is investigating the epidemiology of clinical isolates of *Serratia marcescens* and ESBL-producing *E. coli* under the mentorship of Nicole D. Pecora and Dwight J. Hardy.

Henry F. "Chip" Chambers, MD
Professor of Medicine, Emeritus
Clinical Research on Antibiotic Resistance: Antimicrobial Resistance Leadership Group

Dr. Chambers graduated from Vanderbilt University School of Medicine in 1977. He trained in Internal Medicine and Infectious Diseases at the University of California San Francisco. He was also a Kaiser Foundation Fellow in General Internal Medicine at UCSF and a post-doctoral research fellow at Rockefeller University. Dr. Chambers is Professor of Medicine, Emeritus at UCSF. He served as Chief of Infectious Diseases at San Francisco General Hospital from 1992-2013 and Director of the UCSF Infectious Diseases Fellowship Training Program from 2002-2013. He is a Fellow of the Infectious Diseases Society of America (IDSA) and of the American College of Physicians and was elected to membership in the American Society of Clinical Investigation and the American Association of Physicians. He is editor for the Sanford Guide to Antimicrobial Therapy and he has over 250 publications and textbook chapters in the areas of drug resistance, endocarditis, bacterial infections, and staphylococcal diseases. He is a reviewer for numerous medical publications. He has been a member of advisory groups for the Centers for Diseases Control and Prevention, a chair and reviewer for NIH study sections, and a member of the IDSA treatment guidelines committees. He is a past member of the IDSA board of directors. He and Dr. Vance Fowler are Co-Principal Investigators of the Antibacterial Resistance Leasdership Group. His clinical and research interests are antimicrobial drug resistance, staphylococcal infections, experimental therapeutics, and epidemiology and pathogenesis of disease caused by community methicillin-resistant Staphylococcus aureus.

Andrea T. Cruz, MD, MPH, FAAP
Associate Professor
Pediatrics
Pediatrics Surviving Sepsis Campaign

Andrea Cruz is an associate professor of pediatrics at Baylor in Houston. She received her undergraduate degree from Harvard and her medical degree from Vanderbilt. She completed fellowships in pediatric emergency medicine and pediatric infectious diseases at Baylor and an MPH in epidemiology and global health at the University of Texas. She is the director of research for pediatric emergency medicine at Baylor and is the PECARN site PI for Texas Children's Hospital. Andrea is also the director of the Children's TB clinic at Texas Children's Hospital, the largest US pediatric TB clinic in the country. Andrea serves as a national TB consultant to the CDC and has assisted the World Health Organization and ministries of health with pediatric tuberculosis guidelines. She is also the co-chair of the PEM Special Interest Group of the American Pediatric Association and is an associate editor of Pediatrics. Her clinical and research interests include childhood TB, rapid viral diagnostics, and early recognition of sepsis in the ED.

Sarah Doernberg, MD, MAS
Associate Professor
Clinical Medicine
Diagnostics MASTER-GC

Sarah Doernberg is an Associate Professor in the Division of Infectious Diseases and the Medical Director of Adult Antimicrobial Stewardship at UCSF Medical Center. She has an active outpatient Infectious Diseases Clinic and sees inpatients on the Transplant Infectious Diseases service. Her clinical research focuses on healthcare-acquired infections and antimicrobial stewardship.

She received her BA from Harvard University in Biology with a focus in Neurobiology and received her MD from Yale University School of Medicine. She completed her Internal Medicine residency and Infectious Diseases fellowship at UCSF. She also received a Masters' degree in advanced studies through the Training in Clinical Research Program at UCSF.

Scott Flanders, MD
Professor
Hospital Medicine

Hospitalists Role in Antimicrobial Stewardship

Scott A. Flanders, M.D. is currently Chief Clinical Strategy Officer for Michigan Medicine, Professor in the Division of Hospital Medicine at the University of Michigan, where he serves as Vice Chair for the Department of Internal Medicine. He was the founding leader of Michigan's Hospital Medicine Program, and from 2003-2017 grew the program from four faculty to over 100, while concurrently developing robust clinical, educational, quality and research programs within the section. Dr. Flanders was a founding member of the Board of Directors of the Society of Hospital Medicine (SHM) and is a Past-President of SHM. In 2013, Dr. Flanders was awarded the designation of Master in Hospital Medicine by the Society of Hospital Medicine.

His research interests include hospitalists, hospital-acquired conditions and their prevention, dissemination of patient safety and quality improvement practices, and the diagnosis and treatment of lower respiratory infections. Dr. Flanders developed and leads the Michigan Hospital Medicine Safety (HMS) Consortium, focused on preventing adverse events in hospitalized patients. He has authored over 150 journal articles and book chapters, and has edited two textbooks and a book series in the field of Hospital Medicine.

Vance Fowler, MD, MHS
Florence McAlister Distinguished Professor of Medicine
Clinical Trials on Antibiotic Resistance

Vance Fowler, MD, MHS, Departments of Medicine and Molecular Genetics & Microbiology, Duke University Medical Center. Dr. Fowler is the Florence McAlister Distinguished Professor of Medicine. He has over 2 decades of continuous support as PI from the NIH for clinical and translational research in Staphylococcus aureus and other bacterial infections. Dr. Fowler created the S. aureus Bacteremia Group, co-founded the International Collaboration on Endocarditis, and has been the Contact PI of the Antibacterial Resistance Leadership Group since its inception in 2014. He has over 300 peer-reviewed publications with > 23,000 citations.

Danielle A. Garsin, PhD
Professor
Microbiology and Molecular Genetics
Talk Title TBA

Dr. Garsin is a professor in the McGovern Medical School, Department of Microbiology and Molecular Genetics at the University of Texas Health Science Center at Houston. Dr. Garsin came to UTHealth as an assistant professor in 2004 following a postdoctoral fellowship at Massachusetts General Hospital/Harvard Medical School. She earned her Ph.D. in Biochemistry at Harvard University and her B.S. in Biological Sciences at Cornell University.

Dr. Garsin is at heart a bacterial geneticist, and this foundation supports her interests in bacterial pathogenesis, gene regulation, host-microbe and microbe-microbe interactions. Her studies are centered on the biology of the human bacterial pathogen, Enterococcus faecalis. One NIH-funded research focus is on the roles and regulation of ethanolamine utilization in E. faecalis. Another is on the biology of reactive oxygen species in the immune response elicited in the model host Caenorhabditis elegans. Finally, Dr. Garsin studies the interactions between E. faecalis and the human fungal pathogen, Candida albicans. She and her collaborators discovered that the microbes inhibit each other's virulence leading to the identification of compounds with potential for anti-infective therapeutic development.

Dr. Garsin has received many commendations for excellence in research and education. In 2004, she received an Ellison Medical Foundation New Scholar Award in Global Infectious Disease, and in 2008, she was awarded a UT Young Investigator award. The University of Texas System awarded Dr. Garsin a STAR Award in 2017, the American Academy for Microbiology

elected her as a Fellow in 2019, and McGovern's Women Faculty Forum bestowed an Excellence in Research Award in 2020. For her contributions to education, Dr. Garsin was the recipient of the Dean's Teaching Excellence Award in multiple years. Dr. Garsin is currently serving as a permanent member of the Innate Immunity and Inflammation (III) NIH review group following a term on Prokaryotic Cell and Molecular Biology (PCMB). She is also an associate editor of PLOS Genetics and the minireview editor for mBio.

Thomas P. Giordano, MD, MPH
Professor and Section Chief
Infectious Diseases
HIV Treatment and Resistance

Dr. Giordano is Chief of Infectious Diseases and Professor of Medicine, Baylor College of Medicine, and Investigator, Center for Innovations in Quality, Effectiveness and Safety (IQuESt) at the Michael E. DeBakey VA Medical Center, in Houston, Texas. He is also Medical Director of HIV Services for the Harris Health System, including the Thomas Street Health Center, in Houston. He earned his medical degree from the Johns Hopkins University School of Medicine, completed residency in internal medicine at the Hospital of the University of Pennsylvania, and completed infectious diseases fellowship at Baylor College of Medicine. Dr. Giordano is a board-certified practicing physician who has provided outpatient and inpatient care for adults with HIV since 1999. Dr. Giordano has been Medical Director of the Thomas Street Health Center, one of the largest HIV clinics in the United States (about 6000 current patients), since 2004. Dr. Giordano is an expert in health services research in HIV/AIDS, with a focus on improving retention in HIV care and adherence to care, especially in traditionally underserved populations in the United States. He has led research and demonstration projects funded by the NIH, CDC, HRSA, and VA on retention in HIV care. He is a former member of the FDA Antiviral Drug Advisory Committee and the NIH study section Behavioral and Social Consequences of HIV/AIDS. He is a scientific member of the US Department of Health and Human Services' Panel on Antiretroviral Guidelines for Adults and Adolescents.

Matthew B. Goetz, MD
Chief
Infectious Diseases
The Veteran Health System Experience

Dr. Goetz has substantial experience and expertise in the development, implementation and evaluation of quality improvement programs as well as a broad and deep clinical and investigatory background in Infectious Diseases. In particular, he has been extensively engaged in research related to evaluation of effective antibiotic use and implementing and evaluating operational interventions to improve the quality of antimicrobial use and the quality of care for HIV- and HCV- infected patients. The current application builds logically on my prior work, leverages his local and national roles within the Veterans Health Administration, and makes use of insights gained as a former member of the FDA Anti-infective Drug Advisory Committee. Of note, he is Chief of Infectious Diseases at the VA Greater Los Angeles Healthcare System as well as a member of the VA Infectious Diseases Field Advisory Committee, Antimicrobial Stewardship Task Force (Chair, Implementation Subcommittee), Medication Advisory Panel, Expert Advisory Group for Carbapenem Resistant Enterobacteriaceae, and the HIV Technical Advisory Groups.

Blake Hanson, PhD
Assistant Professor
Epidemiology, Human Genetics & Environmental Sciences

Genomics of Carbapenem-resistant Enterobacterales in the USA

Dr. Hanson is an infectious disease epidemiologist with extensive experience in applying advanced genomic technologies and big-data analytical methods to investigate infectious diseases of public health importance. His laboratory uses a combination of existing and innovative laboratory techniques, and cutting-edge sequencing and bioinformatics to study infectious disease transmission and colonization, how microbial communities impact the development of disease, and how antimicrobial resistance develops and transmits through society.

University of Texas Health Science Center, School of Public Health

Anthony D. Harris, MD, MPH
Professor
Epidemiology & Public Health

ARLG's Role in Supporting Future Generations of ID Investigators

Dr. Harris is an infectious disease physician and epidemiologist whose research interests include emerging pathogens, antimicrobial-resistant bacteria, hospital epidemiology/infection control, epidemiologic methods in infectious diseases and medical informatics. He has published over 230 papers. He has current or has had funding from the NIH, CDC and AHRQ to study antibiotic resistance and hospital epidemiology. He is extremely proud of his mentoring track-record.

University of Maryland

Lee H. Harrison, MD
Professor
Medicine and Epidemiology

Whole Genome Sequencing Surveillance for Prevention of Antibiotic-Resistant Infections

Lee H. Harrison, MD, is Professor of Medicine in the School of Medicine and Professor of Epidemiology in the Graduate School of Public. He is the PI of the Microbial Genomic Epidemiology Laboratory. Dr. Harrison did his undergraduate studies at the University of Pennsylvania and received his medical degree from the Emory University School of Medicine. He completed his internship and residency in internal medicine at the University of Virginia Hospital in Charlottesville. He served as an Epidemic Intelligence Service Officer in the former Meningitis and Special Pathogens Branch of the Centers for Disease Control and Prevention and then completed a fellowship in infectious diseases at Emory.

Dr. Harrison's research focuses on the epidemiology and genomic epidemiology of vaccine-preventable and other serious bacterial infections, including *Neisseria meningitidis* and *Streptococcus pneumoniae.* A current focus of his research is an NIH-funded study that uses bacterial genomics and data mining of the electronic health record to enhance outbreak detection in hospitals. He is also PI the CDC-funded Maryland Active Core surveillance site, which he established in 1991. He is program director of three NIH training grants, a T32 on antimicrobial resistance and international training grants in Mozambique on HIV infection and public health genomic epidemiology in South Africa. He completed a four-year term as a voting member of CDC's Advisory Committee on Immunization Practices in 2016.

Thomas L. Holland, MD
Associate Professor
Medicine, Division of Infectious Diseases
DOTS Study

Dr. Thomas L. Holland, MD, MSc-GH, is an Associate Professor of Infectious Diseases at Duke University and a faculty member of the Duke Clinical Research Institute. His research interests include antibacterial trials, particularly for S. aureus bacteremia and antibiotic-resistant pathogens, as well as the design and implementation of novel clinical trial endpoints including ordinal outcomes and quality of life measures. He is active in COVID clinical care and research.

Robert Jenq, MD
Associate Professor
Genomic Medicine and Stem Cell Transplantation and Cellular Therapy
Microbiome, Bacteria and Cancer

Robert Jenq, MD is Deputy Chair of the Department of Genomic Medicine, an Associate Professor in the Departments of Genomic Medicine and Stem Cell Transplantation and Cellular Therapy, and Director of the Microbiome Core Facility at MD Anderson Cancer Center. He received his medical training at Oregon Health and Science University, Duke, and Memorial Sloan Kettering Cancer Center. A practicing medical oncologist, his research efforts have focused on studying the effects of the intestinal bacterial flora on outcomes following immune therapy for cancer, including hematopoietic cell transplantation and checkpoint blockade.

Zane Laughlin
Graduate Student

Mechanism of 50S Ribosomal Subunit Recognition and Modification by the Mycobacterium tuberculosis rRNA Methyltransferase TlyA

Zane Laughlin is originally from Forest, VA and received his Bachelor's degree in Chemistry with a specialization in Biochemistry from the University of Virginia in 2016. During that time, he worked in the lab of UVA professor Dr. Charles Grisham and as a laboratory technician for Lewis and Clark Pharmaceuticals, Inc. In 2016, he also joined the Biochemistry, Cell and Developmental Biology Graduate Program at Emory University and in 2017 began his thesis work in the lab of Dr. Graeme Conn. Zane's project is focused on understanding the mechanism of recognition and modification of dual-substrate ribosomal methyltransferase TlyA of Mycobacterium tuberculosis. The rRNA modifications introduced by TlyA are necessary for capreomycin binding to the ribosome and thus mycobacterial susceptibility to this second line drug.

Maribel Leon
Graduate Student

Phenotypic and Genotypic Characterization of Viable Bacteria from Cattle Feedyard Dust

Ms. Maribel Leon is a Ph.D. student with an emphasis in epidemiology, Master of Science from the College of Veterinary Medicine & Biomedical Sciences, Texas A&M University and DVM from the National University of Colombia.

Her professional goal is to continue contributing to improving food security through the promotion of health and productivity of animals, all while maintaining public health through improved food safety. In her Ph.D. program, she will continue investigating the dissemination of pathogens and their antimicrobial resistance (AMR) mechanisms, which are responsible for the dramatic decrease of the effectiveness of antimicrobial treatments increasing morbidity, mortality, and expenses of health care.

Anthony Maresso, PhD
Associate Professor
Molecular Virology and Microbiology
Pathobionts and The Gut

Dr. Anthony William Maresso is Associate Professor of Molecular Virology and Microbiology at Baylor College of Medicine. His research program spans the range of vaccine development, the molecular basis of bacterial virulence, biomimetics for infectious disease models, mechanisms of mutagenesis, and evolvable antibacterials. He is the recipient of numerous R01, U19, and R21 NIAID grants, has published greater than 50 original peer reviewed articles, and is the author of the textbook “Bacterial Virulence: A Conceptual Primer”. He also founded TAILOR LABS, a BCM initiative engaged in developing phage for use in antibiotic-resistant infections.

William R. Miller, MD
Assistant Professor
Division of Infectious Diseases
Center for Antimicrobial Resistance and Microbial Genomics (CARMiG)

Specific Host-pathogen Interactions of Enterococcus Faecalis

William R. Miller, M.D. is an assistant professor with the Division of Infectious Diseases and a member of the Center for Antimicrobial Resistance and Microbial Genomics (CARMiG) at the University of Texas McGovern Medical School (UTHealth). He received his medical degree and completed his residency training in Internal Medicine/Pediatrics at UTHealth. He completed a fellowship in adult Infectious Diseases at McGovern Medical School at UTHealth and the UT MD Anderson Cancer Center program. Dr. Miller's current research interests involve the clinical impact and mechanistic bases of antimicrobial resistance. Active projects include studying the multilayered cell membrane defense networks of Gram-positive pathogens using enterococci as model organisms, understanding the inoculum effect in severe methicillin-sensitive Staphylococcus aureus infections and characterizing the molecular mechanisms of resistance of multidrug resistant Gram-negative bacteria.

Jose M. Munita, MD
Associate Professor

Challenging Clinical Cases on Antimicrobial Resistance: Susceptibility Interpretation

Jose M. Munita is a physician scientist currently working at Clinica Alemana – Universidad del Desarrollo in Santiago, Chile, where he holds a position as an Associate Professor. Dr. Munita received his medical degree from Universidad de los Andes, Santiago, Chile in 2004 and trained as an Internal Medicine specialist in Clinica Alemana, also in Santiago, Chile. He then moved to the US where he completed an Infectious Diseases fellowship at the University of Texas McGovern Medical School and the MD Anderson Cancer Center, Houston, TX. After his training, he moved back to Chile where he is currently working. Dr. Munita's group is studying the genomic evolution of the Chilean-Cordobes clone of methicillin-resistant S. aureus and he is currently leading a country wide effort to improve the knowledge about antimicrobial resistant organisms in Chile.

Flor Muñoz-Rivas, MD
Associate Professor
Pediatrics-Infectious Disease
Immunization Panel Discussion

Dr. Muñoz is an Associate Professor of Pediatrics, Infectious Diseases, Molecular Virology and Microbiology at Baylor College of Medicine, and Director of Transplant Infectious Diseases at Texas Children's Hospital, Houston, TX. She is a physician-scientist with projects focusing on the epidemiology of respiratory infections in healthy and immunocompromised hosts, and the evaluation of vaccines in pregnant women and children. She also serves as chair of the Institutional Review Board at Baylor College of Medicine. Dr Muñoz is a member of the American Academy of Pediatrics (AAP) Committee on Infectious Diseases (COID), the Influenza Work Group of the CDC's Advisory Committee on Immunization Practices (ACIP), the American College of Obstetricians and Gynecologists (ACOG) Immunization Expert Work Group, and the Pediatric Infectious Diseases Society and the European Society of Pediatric Infectious Diseases. She is Editor of the Maternal Neonatal Section of the Pediatric Infectious Diseases Journal, co-Chair of the Maternal Immunization working group of COVAX-CEPI, and Chair of the Maternal Immunization working group of the NIH IDCRC.

Daniel Musher, MD
Distinguished Service Professor
Medicine-Infectious Disease
Panel: *Clinical Cases*

Dr. Daniel Musher is a Distinguished Service Professor of Medicine and Professor of Molecular Virology and Microbiology at the Baylor College of Medicine and has been on staff at the VA Medical Center Houston since 1971. His has worked in the classical triad of clinical medicine, teaching and research. His research has focused on bacterial infections, principally those due to Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae and Treponema pallidum; he is the coauthor of 570 papers and chapters.

Dr. Musher was the founding concertmaster of the Texas Medical Center Orchestra. He plays string quartets weekly and otherwise reads for recreation. He is active in communal organizations including the boards of trustees of the Jewish Community Center, Bureau of Jewish Education, Hillel Foundation (past president), Jewish Federation of Greater Houston, Congregation Beth Yeshurun (pas vice-president), Beth Yeshurun Schools (Past President) and Chamber Music Houston (past President). He and his wife, Karol, have three married children and ten grandchildren.

Robin Patel, MD
Professor
Medicine and Microbiology

Whole Genome Sequencing and Detection of Resistance in the Routine Clinical Laboratory

Dr. Robin Patel is the Elizabeth P. and Robert E. Allen Professor of Individualized Medicine, Professor of Medicine, Professor of Microbiology, Director of the Infectious Diseases Research Laboratory, and Co-Director of the Clinical Bacteriology Laboratory at the Mayo Clinic. She is board certified in Infectious Diseases, as well as Medical and Public Health Microbiology. Dr. Patel is the immediate Past-President of the American Society for Microbiology and a Fellow of the American Academy of Microbiology.

Dr. Patel's research, supported by the National Institutes of Health (NIH), focuses on bacterial resistance, development and utilization of diagnostic tests, and biofilms. She has published over 400 peer-reviewed manuscripts.

In addition to her positions at the Mayo Clinic, Dr. Patel is a member of the NIH's National Institute of Allergy and Infectious Diseases (NIAID) Council, Director of the NIH Laboratory Center for the Antibacterial Resistance Leadership Group (ARLG), an advisor to the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antimicrobial Susceptibility Testing, a member of the American Board of Pathology Medical Microbiology and Clinical Pathology for ABPath CertLink™ Test Development and Advisory Committees, and an associate editor of *Clinical Infectious Diseases*. She is also a past chair of the U.S. Medical Licensing Examination Microbiology and Immunology Test Material Development Committee and a current member of a U.S. Medical Licensing Examination Item Review Group.

Katherine K. Perez, PharmD, BCIDP
Infectious Diseases Clinical Specialist
Pharmacy & Pathology and Genomic Medicine

Update on Rapid Diagnostics

Dr. Perez is an infectious diseases clinical specialist at Houston Methodist and the pharmacy lead for the system's antimicrobial stewardship program. She received her Doctor of Pharmacy degree from the University of Texas College of Pharmacy in Austin, Texas in 2010. Completed a postdoctoral pharmacy practice residency in a combined program at University Health System Hospital and the Pharmacotherapy Education and Research Center at the University of Texas Health Science Center in San Antonio, Texas followed by a specialty residency in infectious diseases pharmacotherapy at Houston Methodist Hospital, Houston, Texas. BCIDP - board certified in infectious diseases pharmacotherapy by the Board of Pharmaceutical Specialties.

Trish M. Perl, MD, MSc
Professor
Internal Medicine
The Role of HAI Prevention and Antimicrobial Resistance

Trish M. Perl, MD, MSc, is the Jay P Sanford Professor in the Departments of Medicine (Infectious Diseases) and the Chief of the Division of Infectious Diseases and Geographic Medicine at UT Southwestern Medical Center in Dallas TX. She is the Chief of Infectious Diseases at Parkland Hospital and Health System and the Interim Associate Medical Director of Infection Prevention there. She formerly was at Johns Hopkins University School of Medicine in the Division of Infectious Diseases in the Department of Medicine, in Epidemiology at the Bloomberg School of Public Health and the Senior Epidemiologist for Johns Hopkins Health System. Dr. Perl received her Bachelor of Arts and medical degree from the University of North Carolina at Chapel Hill and a Master of Science degree from McGill University in Montreal, Canada. She completed a residency in internal medicine at McGill University and a fellowship in infectious diseases and clinical epidemiology at the University of Iowa. She was on faculty at the University of Iowa for several years before moving to Hopkins where she was the hospital epidemiologist from 1996 to 2011 and then the healthsystem epidemiologist until 2016 when she moved to Dallas. She has extensive practical and research experience in the field of healthcare associated infections and resistant and epidemiologically significant organisms and is recognized globally for her innovation and research in healthcare associated infections, antimicrobial resistance, their transmission and prevention.

An active researcher, Dr. Perl has been funded by the CDC and the Veteran's Affairs Administration over the years. She has authored or coauthored over 250 peer-reviewed articles. In addition, she has written

University of Texas Southwestern, Dallas

multiple chapters and contributed to guidelines and policies relevant to healthcare associated infections at the institutional, state and federal level. She serves on NIH study sections and on IOM committees including those for Ebola. She has been asked to help with management of international outbreaks including COVID-19, SARS, MERS CoV, Ebola and consults with international governments on guideline development and strategies to prevent healthcare associated infections and antimicrobial resistance.

David E. Persse, MD FACEP FAEMS
Physician Director
Panel Discussion: The Houston Response to COVID-19 Lessons Learned

Dr. Persse's career in medicine started with ten years experience as a field paramedic and paramedic instructor in upstate New York and New Jersey. After receiving his pre-med training at Columbia University in New York, he then attended Georgetown University School of Medicine. Graduating with honors in emergency medicine from Georgetown, Dr. Persse then completed residency training in emergency medicine at Harbor-UCLA Medical Center in Torrance, California. After residency, Dr. Persse completed a resuscitation research fellowship at the Ohio State University. Dr. Persse was then awarded a grant from the Society for Academic Emergency Medicine and completed fellowship training in emergency medical services and resuscitation at the Baylor College of Medicine. Following his EMS fellowship Dr. Persse became the Assistant Medical Director for the Emergency Medical Services system of Houston. He then returned to California to become the Medical Director of the Los Angeles County Paramedic Training Institute, and the Assistant Medical Director of the Los Angeles County EMS Agency. In 1996 Dr. Persse returned to Houston to assume the role of the Director of Emergency Medical Services for the City of Houston. In May of 2004 he was appointed by City Council as Houston's Public Health Authority. He has been the lead author or co-author on over 50 peer reviewed journal articles.

Dr. Persse is also a member of the Board of Directors and Executive Committee for the South East Texas Trauma Regional Advisory Council and a previous Chair of the National Registry of Emergency Medical Technicians. He is the recipient of the Keith Neely Outstanding Contribution to the National Association of EMS Physicians for his

leadership during the Hurricane Katrina response, 2007, and the 2009 Michael K. Copass Award from the U.S. Metropolitan Medical Directors. He is a 2015 EMS Top 10 Innovator Award Winner from the Journal of Emergency Medical Services. Dr. Persse received an Honorary Doctorate in Humanities in Medicine from Baylor College of Medicine and was awarded the ACEP Outstanding Contribution to EMS Award in 2018. Dr. Persse is a Professor of Medicine and Surgery at the Baylor College of Medicine and Associate Professor of Emergency Medicine at the University of Texas Medical School – Houston. He is also a Tactical Physician with the Houston Police S.W.A.T. team.

Melinda Pettigrew, PhD
Professor
Epidemiology

ARLG's Role in Supporting Future Generations of ID Investigators

Melinda Pettigrew, PhD, is a Professor of Epidemiology (Department of Epidemiology of Microbial Diseases), the Senior Associate Dean for Academic Affairs, and Deputy Title IX coordinator at the Yale School of Public Health. Professor Pettigrew's research focuses on pathobionts of the respiratory and gastrointestinal tracts (e.g., Haemophilus influenzae and Pseudomonas aeruginosa) and the public health threat of antibiotic resistance. Her current work utilizes an interdisciplinary approach involving infectious disease epidemiology and microbiology to identify factors that influence whether pathobionts asymptomatically colonize or cause diseases such as pneumonia and exacerbations of chronic obstructive pulmonary disease (COPD). Additional projects utilize next-generation sequence technologies (e.g., whole-genome sequencing, 16S rRNA gene microbial profiling, and RNA-sequencing) to determine how disruptions of homeostasis in the respiratory and gastrointestinal microbiome influence colonization resistance, development of antibiotic resistance, and risk of both hospital and community acquired infections. She serves on the Steering and Executive Committees for the Antibiotic Resistance Leadership Group (ARLG). As the Associate Director of the Scientific Leadership Core, focusing on Diversity, Professor Pettigrew leads efforts implement and integrate principles of diversity, access, equity, and inclusion throughout the ARLG. She completed a fellowship from the Hedwig van Ameringen Executive Leadership in Academic Medicine (ELAM) Program for Women in 2013 and was a Public Voices Thought Leaders Fellow in 2014-2015. Professor Pettigrew serves on the editorial board of mBio. Professor Pettigrew has a bachelor's degree from Grinnell College and a doctoral degree from the Yale University.

Yale University

Jason M. Pogue, PharmD, BCPS, BCIDP
Clinical Professor
Clinical Pharmacy

Refocusing Antimicrobial Stewardship Efforts to Optimize Patient Outcomes

Dr. Jason Pogue is a Clinical Professor in the Department of Clinical Pharmacy at the University of Michigan College of Pharmacy and an Infectious Diseases Clinical Pharmacist at Michigan Medicine. Prior to this position he spent just over a decade at the Detroit Medical Center (DMC) as an infectious diseases clinical pharmacist at Sinai-Grace Hospital and as the co-chair of the Antimicrobial Stewardship Committee at the DMC.

Dr. Pogue received a bachelor degree in Chemistry from Gannon University, before obtaining his doctor of pharmacy degree from the University of Pittsburgh. He then completed a PGY-1 pharmacy residency at the University of Pittsburgh Medical Center followed by an infectious diseases PGY-2 residency at the University of Michigan Health Systems. His research interests focus on epidemiology and management of multi-drug resistant Gram-negative organisms and antimicrobial stewardship.

Dr. Pogue is a recognized leader in Gram-negative resistance and antimicrobial stewardship as evidenced by his significant contribution of over 100 peer-reviewed articles, over 100 abstracts, multiple book chapters, and presentations at numerous national and international conferences. Dr. Pogue currently serves at the immediate past president of the Society of Infectious Diseases Pharmacists and is an active member of The United States Committee on Antimicrobial Susceptibility Testing (USCAST) where he is intimately involved with antimicrobial susceptibility breakpoint setting. Dr. Pogue also serves as the clinical pharmacy lead for two National Institutes of Health (NIH) funded international studies targeting strategies to optimize polymyxin usage.

University of Michigan

Eva Preisner, PhD
Postdoctoral Associate

Simplified Microbial Communities as a Safe Antimicrobial Treatment Option in Clostridioides difficile infections

Dr. Eva Preisner conducted her undergraduate studies in Water Sciences at the University of Duisburg-Essen in Germany. After moving to the United States, she linked her passion for the environment with her interest in public health and studied at The Arnold School of Public Health at the University of South Carolina, where she received her MS and Ph. D.

Dr. Preisner joined the Microbial Therapeutic Laboratory under Dr. Britton at Baylor College of Medicine in 2018 as a postdoctoral researcher and has been a trainee in the Training Program for Antimicrobial Resistance since 2020. Her current research interest is developing microbial therapeutics to prevent and treat gastrointestinal diseases as an alternative to antibiotic use.

Hans-Georg Sahl, PhD
Emeritus Professor
Pharmaceutical Microbiology

Novel Insights into the Mechanism of Daptomycin Action

Hans-Georg Sahl studied Biology and Chemistry and earned a PhD in Microbiology from the University of Bonn. He was Associate Professor of Medical Microbiology in Bonn since 1990 and became Professor of Medical and Pharmaceutical Microbiology in 2004; he retired 2018. His research group focused on bacterial cell wall biosynthesis using staphylococci and chlamydia as model organisms, and mechanisms of antibiotic action of cell wall active natural compounds, including antimicrobial host defense peptides, lantibiotics and glycopeptides. He was coordinator of several major research consortia such as the specific research unit “Post genomic strategies for Novel Antibiotic Drugs and Targets” sponsored by the German Research Foundation (2008 – 2014) and the translational unit “Novel Anti-infectives” of the German Centre of Infection Research (DZIF, 2012 – 2016). He served as Co-chair of the Gordon Research Conferences on “Antimicrobial Peptides” (2003) and “New Antibiotics Discovery and Development” (2012).

Michael Satlin, MD
Associate Professor
Medicine
Trialist in Training

Dr. Satlin received his M.D. from the University of Virginia and completed residency training in Internal Medicine and fellowship training in Infectious Diseases at Weill Cornell Medical College. He also received a Master's Degree in Clinical and Translational Investigation at Weill Cornell.

He is Clinical Director of the Transplantation-Oncology Infectious Diseases Program at Weill Cornell and provides infectious diseases supportive care to immunocompromised hosts. Dr. Satlin's research interests are in the epidemiology, diagnosis, and treatment of multidrug-resistant Gram-negative bacterial infections in immunocompromised hosts, and his research is supported by grants from NIAID and industry. He is Co-Chair of the Breakpoint Working Group of the Clinical and Laboratory Standards Institute's Subcommittee on Antimicrobial Susceptibility Testing and participates on multiple committees of NIAID's Antibacterial Research Leadership Group. He also serves as Associate Editor for *Journal of Antimicrobial Chemotherapy-Antimicrobial Resistance* and on the Editorial Advisory Boards of *Clinical Infectious Diseases* and *Open Forum Infectious Diseases*.

Edward J. Septimus, MD, FIDSA, FACP, FSHEA

Professor

Panel: Clinical Cases

Dr. Ed Septimus received his medical degree from Baylor College of Medicine in Houston in 1972. Dr. Septimus went on to complete his postgraduate training in internal medicine and infectious diseases at Baylor College of Medicine in Houston. Dr. Ed Septimus is board certified in both internal medicine and infectious diseases. He was VP Research and Infectious Diseases HCA Healthcare until 2018. Prior to HCA, he was the medical director of infectious diseases and occupational health for Memorial Hermann Healthcare System. He has served on the Board of Directors of the Infectious Diseases Society of America (IDSA) and was on the IDSA Antimicrobial Resistance Committee, the SHEA Antimicrobial Stewardship Committee, and the IDSA Quality Measurement Committee. He was the first recipient of the IDSA Watanakunakorn Clinician Award. In 2011 he was appointed to the Healthcare-Associated Infections/Preventable Adverse Events Advisory Panel for the Texas Department of State Health Services. He was awarded the John S Dunn Sr. Outstanding Teacher Award in 2010, 2011, 2013 and 2014. He is on the FDA Anti-Infective Drug Advisory Group and is co-chair of the NQF Patient Safety Steering Committee. He holds a faculty position as Adjunct Professor at Texas A&M College of Medicine, Senior Lecturer Department of Population Medicine Harvard Medical School, and Professor Distinguished Senior Fellow, School of Public Health, George Mason University. He has published over 120 articles and chapters.

Harvard University and Texas A&M College of Medicine, Houston

Bhavarth Shukla, MD
Assistant Professor
Clinical Infectious Disease

An outbreak of Candida auris During a COVID-19 Case Surge in Florida

Dr. Bhavarth Shukla completed his medical degree from Medical University of SC and public health degree from UNC- Chapel Hill. He completed his residency training in internal medicine at University of Miami and fellowship training in ID at UT Health Science Center at Houston. He is presently assistant professor of clinical infectious disease at the University of Miami and serves as the Medical Director for Infection Control for UHealth and Associate Program Director for the Internal Medicine Residency Program. His research interests include antimicrobial resistance, infection control and more recently the intersection of these in the context of the COVID-19 pandemic.

Pranita D. Tamma, MD, MHS
Associate Professor
Pediatrics

Detecting ESBL-carrying Gram-negative Bacteria: the Aftermath of MERINO

Dr. Tamma an Associate Professor of Pediatrics and the Director of the Pediatric Antimicrobial Stewardship Program at The Johns Hopkins University School of Medicine. She is also an Associate Professor in the Department of Epidemiology at The Johns Hopkins Bloomberg School of Public Health. Her research focuses on: (a) elucidating mechanisms of resistance in Gram-negative organisms (including both the Enterobacterales and glucose non-fermenting bacteria), (b) developing and enhancing rapid phenotypic and genotypic methods to identify Gram-negative resistance to enable critically-ill patients to be placed on appropriate antibiotic therapy as early as possible, and (c) identifying optimal treatment strategies for patients infected with multidrug-resistant Gram-negative infections, including leading a multicenter trial of bacteriophage therapy to treat Pseudomonas aeruginosa infections. She past and present funding from the NIH, FDA, CDC, and AHRQ to investigate these areas. She has had the opportunity to work in the national and international arena to advance her understanding of Gram-negative resistance including: serving as an Editor at Antimicrobial Agents and Chemotherapy and The Journal of the Pediatric Infectious Diseases Society; serving as 1 of 12 international voting members of the Clinical Laboratory and Standards Institute that provides international guidance on phenotypic and genotypic methods for identifying antimicrobial resistance; serving as a voting member of the NIH-funded Antibacterial Resistance Leadership Group Gram-Negative Resistance Subcommittee; and serving as the Co-Chair for the Infectious Diseases Society of America Antimicrobial Resistance Guidance. She is the Co-Director of the Hopkins Integrated Center for Combating Antimicrobial Resistance. She is very passionate about furthering the science of antimicrobial resistance and to continue to work with and learn from leaders in the field.

Aimee Tierney
Graduate Student
Characterization of a LysR Regulator in MDR A. baumannii

Aimee Tierney is currently a 5th year doctoral student in Emory University's Microbiology and Molecular Genetics program. She is a member of Philip Rather's laboratory, where she studies the regulation of a virulence switch between opaque and translucent colony variants in the bacterial pathogen Acinetobacter baumannii. Her focus is the characterization of a LysR-type transcriptional regulator that impacts pathways in the virulence switch, quorum sensing, and motility.

Ephraim Tsalik, MD, PhD
Associate Professor
Medicine and Molecular Genetics & Microbiology
RADICAL Study

Ephraim Tsalik is an Associate Professor in the Department of Medicine and the Department of Molecular Genetics & Microbiology at Duke University. He earned his MD and PhD degrees at Columbia University. He then came to Duke for training in Internal Medicine and Infectious Diseases, after which he joined the faculty. Dr. Tsalik also practices emergency medicine at the Durham VA Health Care System and serves as the Associate Director of the ARLG Laboratory Center. In his research capacity, Dr. Tsalik evaluates existing and emerging biomarkers, supports the development of diagnostic platforms for pathogen identification and characterization, and has supported a new paradigm for host response-based diagnostics.

Netanya Utay, PhD
Associate Professor
General Internal Medicine
Microbiome Transplantation in Chronic Diseases

Dr. Netanya Utay is an associate professor at UT Health Science Center at Houston. She attended medical school at Baylor College of Medicine and trained in internal medicine at the University of Washington and infectious diseases at the National Institutes of Health. She joined the faculty at the University of Texas Medical Branch at Galveston in 2013 before being recruited to UT Health Science Center at Houston to join the Division of General Medicine, Department of Medicine in July 2017. She attends on both internal medicine and infectious diseases and maintains a busy outpatient HIV clinic. Dr. Utay's research interests include evaluating the contributions of gut damage, the microbiome, and inflammation to the development and progression of chronic diseases including HIV, Parkinson's disease, and non-alcoholic fatty liver disease. Dr. Utay serves as the Deputy Director for the Kelsey Research Foundation – University of Texas Health Science Center Microbiome Research Program and as Vice-Chair of the End-organ Disease and Inflammation Transformative Science Group of the AIDS Clinical Trials Group. She has conducted numerous studies aiming to modulate inflammation and restore a healthy microbiome.

Luis Vega, PhD
Post-doctoral Research Fellow
Pediatrics

Contribution of a Mobile Genetic Element to Adherence, Invasion and Pathogenesis of Emergent Antimicrobial Resistant Group A Streptococcus

Luis Vega obtained his B.A. from Rice University in 2005 and his PhD in Molecular Biology and Microbial Pathogenesis from Washington University in St Louis School of Medicine in 2012. He has studied the pathogenesis mechanisms of the Group A Streptococcus throughout his career, first under his graduate mentor, Dr. Michael Caparon, then as a postdoctoral fellow under Dr. Kevin McIver and now in the lab of Dr. Anthony Flores in the Pediatrics Department of the McGovern Medical School. Currently, he studies the association of antimicrobial resistance in Group A Streptococcus with disease phenotypes and mobile genetic element-encoded virulence factors.

Minggui Wang, MD, FESCMID, FISAC
Professor
Medicine

“All of Us are Better Than Any of Us”: Early Insights in Global Clinical and Molecular Epidemiology of Carbapenem-Resistant Enterobacterales

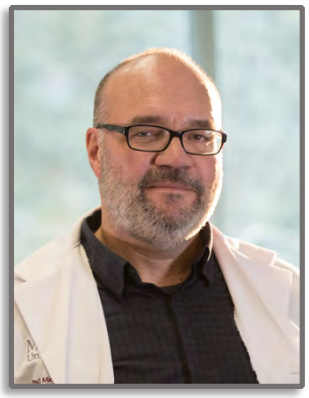
Dr. Wang is a Professor of Medicine of Fudan University, Chief-in-Physician, Director of Institute of Antibiotics, and Vice Director, Division of Infectious Diseases, Huashan Hospital, Fudan University. His clinical work focus on diagnosis and treatment of bacterial and fungal infections, and rational use of antimicrobials in clinical practice. His research interests are bacterial resistance and mechanisms of bacterial resistance. Dr. Wang is the president of Committee of Society on Bacterial Infection and Resistance, Chinese Medical Association. He is an Executive Committee Member of ISAC, and an ESCMID fellow and ISAC fellow.

Fudan University

David S. Weiss, PhD
Associate Professor
Medicine/Div. of Infectious Diseases
Director, Emory Antibiotic Resistance Center
Heteroresistance in Gram-negative Bacteria

Dr. Weiss received his PhD in Microbiology from New York University in 2004. Working under Dr. Arturo Zychlinsky, he studied how Toll-like Receptors work together to fight bacterial infections. He completed his postdoctoral training at Stanford University under the guidance of Drs. Stanley Falkow and Denise Monack, studying virulence mechanisms of Francisella and the role of the inflammasome in host defense. He was the recipient of a three year postdoctoral fellowship from the Giannini Family Foundation.

Since starting his lab at Emory University in 2008, Dr. Weiss has continued to study the ways in which bacteria cause disease and subvert the host immune response, as well as how they resist antibiotics. In 2013, Dr. Weiss was named a Burroughs Wellcome Fund Investigator in the Pathogenesis of Infectious Disease. He also won the Albert E. Levy Scientific Research Award at Emory in 2015 for his work elucidating a novel role for the Cas9 protein in gene regulation. His current research is focused on the role of antibiotic heteroresistance in causing treatment failures as well as how heteroresistance can be exploited to rationally design effective combination therapies. Dr. Weiss is the director of the Emory Antibiotic Resistance Center.



Gerry Wright, PhD
Professor
Biochemistry and Biomedical Sciences
Discovering New Antibiotics: State of the Art

Gerry Wright is the Director of the Michael G. DeGroote Institute for Infectious Disease Research and the David Braley Centre for Antibiotic Discovery. He is a Professor in the Department of Biochemistry and Biomedical Sciences at McMaster University and holds the Michael G. DeGroote Chair in Infection and Anti-Infective Research and a Tier 1 Canada Research Chair in Antibiotic Biochemistry. From 2001-2007 Gerry served as Chair of the Department of Biochemistry and Biomedical Sciences at McMaster. Gerry was elected as a Fellow of the Royal Society of Canada (2012) and a fellow of the American Academy of Microbiology (2013). He is the recipient of a Killam Research Fellowship (2011-1012), R.G.E. Murray Award for Career Achievement of the Canadian Society of Microbiologists (2013), and the NRC Research Press Senior Investigator Award from the Canadian Society for Molecular Biosciences (2016), Premier's Research Excellence (1999) and the Polanyi Prize (1993). He is the co-founder of the Canadian Anti-Infective Innovation Network (www.cain-amr.ca). He has trained over 70 graduate students and postdocs, is the author of over 275 manuscripts and is a member of the editorial boards of several peer-reviewed journals. Gerry is the co-founder of Symbal Therapeutics. In 2016 he was named a McMaster Distinguished University Professor, the highest academic honor at the university. His research interests are in the origins and mechanisms of antibiotic resistance and the discovery of new anti-infective strategies, in particular focusing on the application of microbial natural products and synthetic biology towards this goal.

University of McMaster

Rapid Fire Presenters

Day 1

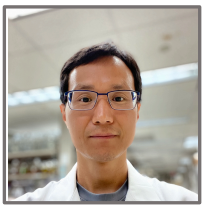
Alex Deyanov, Graduate Student, Rice University
Pyoverdine Antivirulents Synergize with Gallium Nitrate to Inhibit Pseudomonas aeruginosa
Poster 19

Abiola Olaitan, Postdoc, IBT, Texas A&M
Cellular Response of C. difficile Epidemic 027 to Metronidazole-induced Oxidative Stress
Poster 20

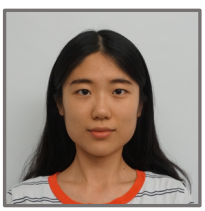
Diana Panesso, Assistant Professor, UT Health Science Center Houston
Role of the LiaF in the LiaR-Mediated Response Against Daptomycin and Antimicrobial Peptides in Multidrug-Resistant Enterococcus faecalis (Efs)
Poster 21

Paul "Skip" Price, Assistant Professor, Eastern Michigan University
Using Complex Microbial Communities to Identify Microbes with Cryptic Antibiotic Potential
Poster 22

Cecilia Sierra-Bakhshi, Graduate Student, Marshall University
The MacAB Efflux Pump is Involved in Protecting Serratia marcescens from Aminoglycoside Antibiotics, but not from Macrolide Antibiotics
Poster 23



Zhizeng Sun, Assistant Professor, Baylor College of Medicine
Deciphering the Determinants of KPC-2 Carbapenemase Activity and Substrate Specificity Using Random Mutagenesis and Deep Sequencing
Poster 24



Yue Zhou, Graduate Student, Rice University
Structural and Biochemical Studies of MurAA, an Enolpyruvate Transferase that Contributes to Cellular Fitness During Daptomycin Attack in Enterococcus faecium
Poster 25

Rapid Fire Presenters

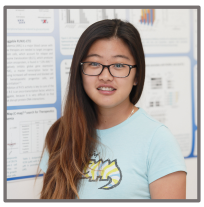
Day 2

Mario Arrieta-Ortiz, Assistant Professor, Baylor College of Medicine
Postdoc, Institute for Systems Biology
Poster 43

Sara Gomez-Villegas, Postdoc, UT Health Science Center Houston
Amino Acid Substitutions in Key Regions of BlaI and BlaR Correlate with the Cefazolin Inoculum Effect in Methicillin Susceptible Staphylococcus aureus (MSSA)
Poster 44

RaviKanthReddy Marreddy, Postdoc, Texas A&M IBT
Chemical Genetic Exploration of Clostridium difficile Toxin Metabolism, Toward Defining Anti-virulent Drug Targets
Poster 45

Amila Nanayakkara, Postdoc, UT Southwestern
Target Validation of Novel Antibiotic, Peptide-Conjugated Phosphorodiamidate Morpholino Oligomers (PPMOs)
Poster 46



Jane Park, Graduate Student, Baylor College of Medicine
Discovery of Inhibitors of the KPC-2 Carbapenemase Using a Focused DNA-Encoded Library
Poster 47

Keiko Salazar, Graduate Student, Baylor College of Medicine
Anti-Viral Resistance and Phage Counter Adaptation to Pandemic E. coli
Poster 48

Charlie Seto, Postdoc, Baylor College of Medicine
Annotation-Agnostic Metagenomic Biomarkers of Infectious Disease Susceptibility
Poster 49

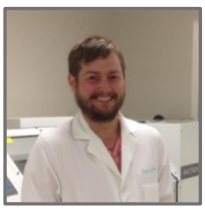
Rapid Fire Presenters

Day 3

Michael Hansen, Assistant Professor, Baylor College of Medicine
Urine Culture High Contamination Rates call into Question the Gold Standard for Urinary Tract Infections
Poster 68

Jonathon McNeil, Assistant Professor, Baylor College of Medicine
Back to The Future: Increasing Penicillin Susceptibility among Methicillin-Susceptible Staphylococcus aureus Osteoarticular Infections in Children
Poster 69

Belkys Sánchez, Postdoc, Baylor College of Medicine
Development of Bacteriophages with Anti-Biofilm Properties as Novel Treatment for Catheter-Associated Urinary Tract Infections
Poster 70



William Shropshire, Graduate Research Assistant, UT Health Science Center Houston
Non-carbapenemase Producing Organisms with CTX-M Gene Amplification Account for Majority of Invasive Carbapenem-resistant Enterobacterales bacteremia in Immunocompromised Patient Population
Poster 72

Jennifer Walker, Assistant Professor, UT Health Science Center Houston
Characterization of the Antimicrobial Susceptibility Patterns and Virulence Mechanisms Promoting Staphylococcal Medical Device Infections
Poster 73

List of Poster Presenters In alphabetical order of presenter

Last Name	First Name	Institution	*Poster Title*	Poster #	Day of Presentation
Alnezary	Faris	University of Houston	*An Invertebrate Model to Study Clostridioides difficile*	1	1
Amirrad	Maryam	University of Ottawa	*Learner Needs Assessment Antimicrobial Stewardship Knowledge of Pediatric Residents in Ontario*	26	2
Arrieta-Ortiz	Mario	Institute for Systems Biology	*Predictive Regulatory and Metabolic Network Models for Systems Analysis of Clostridioides difficile*	43	2
Bevan Rydell	Kristen	University of Texas Health Science Center Houston	*Bacterial Complications in COVID-19 Patients and Trends in Rate of Positive Bacterial Cultures Before and After COVID-19 Pandemic in a Large Single Healthcare System in the Houston Metropolitan Area*	2	1
Bier	Naomi	University of Texas Health Science Center Houston	*Successful Gut Decolonization of Extended-Spectrum β-Lactamase Producing Klebsiella pneumoniae Using Oral Lyophilized Fecal Microbiota Transplant (FMT) In a Woman with Recurrent Urinary Tract Infections*	27	2
Carvajal	Lina	Universidad El Bosque	*Diagnostic Performance of Rapid Test for Detection of Cefazolin Inoculum Effect (CzIE) in Methicillin-Susceptible Staphylococcus aureus recovered from bacteremia in Latin-American hospitals*	51	3
Castro	Betsy	Universidad El Bosque	*Transcriptomic Alterations Associated to Heterogeneous Vancomycin-Intermediate Staphylococcus aureus (hVISA) Phenotype in Latin-American MRSA Isolates*	3	1
Cubria	Maria	University of Texas Health Science Center Houston	*Population Genomics Reveals Distinct Temporal Association with the Emergence of ST1 Serotype V Group B Streptococcus and Macrolide Resistance*	28	2
Dague	Asley	Marshall University	*Evaluation of Antimicrobial Properties of Extracts from the Model Moss Ceratodon purpureus*	52	3

Last Name	First Name	Institution	*Poster Title*	Poster #	Day of Presentation
De la Hoz	Alejandro	University of Texas Health Science Center Houston	*Prevalence of the Cefazolin Inoculum Effect in a Prospective Cohort of Patients with MSSA Bacteremia in Texas and the Impact of Treatment with Cefazolin or Nafcillin*	4	1
De la Hoz Gomez	Alejandro	University of Texas Health Science Center Houston	*The Cefazolin Inoculum Effect in Staphylococcus aureus Bacteremia is Associated with Poor Clinical Outcomes: Results from a Prospective Latin American Bacteremia Study*	29	3
Deyanov	Alex	Rice University	*Pyoverdine Antivirulents Synergize with Gallium Nitrate to Inhibit Pseudomonas aeruginosa*	19	1
Duran Ramirez	Jesus	University of Texas Health Science Center Houston	*Characterization of Antibiotic Susceptibility Patterns and Virulence Mechanisms in Staphylococcus aureus Urinary Catheter-associated Isolates*	53	3
Echeverri	Aura María	Universidad El Bosque	*Genetic Context of Mercury Resistance Genes in Latin-American Staphylococcus aureus Strains*	5	1
Ersoy	Selvi	University of California Los Angeles	*Impact of Bicarbonate on PBP2a Production, Maturation and Functionality in Selected Methicillin-Resistant Staphylococcus aureus (MRSA) Strains*	30	2
Eubank	Taryn	Houston Methodist	*Impact of a Fluid COVID-19 Treatment Algorithm on Clinical Outcomes and Characterization of Disease Progression*	6	1
Gomez-Villegas	Sara	University of Texas Health Science Center Houston	*Management of Staphylococcus aureus Bacteremia Among Adult Infectious Disease Specialists in Latin America: A Wide Variation in Treatment Approaches*	74	3

Last Name	First Name	Institution	*Poster Title*	Poster #	Day of Presentation
Gomez-Villegas	Sara	University of Texas Health Science Center Houston	*Amino Acid Substitutions in Key Regions of BlaI and BlaR Correlate with the Cefazolin Inoculum Effect in Methicillin Susceptible Staphylococcus aureus (MSSA)*	44	2
Green	Sabrina	Baylor College of Medicine	*Tailored Antibacterials and Innovative Laboratories for Phage (F) Research: A New Service Center Developing Personalized Antimicrobials for Vulnerable Patients*	55	3
Guha	Shantanu	University of Texas Health Science Center Houston	*Development of Novel Antifungals Against Candida Based on an Antifungal Peptide Produced by E. faecalis*	7	1
Hansen	Michael	Baylor College of Medicine	*High Contamination Rates in Urine Cultures call into Question the Gold Standard for Urinary Tract Infections*	68	3
Howard-Anderson	Jessica	Emory University	*Increased Mortality with Polymyxins Compared to Ceftolozane/Tazobactam in Carbapenem-Resistant Pseudomonas aeruginosa Infections*	32	2
Hu	Chenlin	University of Houston	*Development of the High Performance Liquid Chromatography to detect vancomycin in clinical human fecal samples*	56	3
Jaggavarapu	Siddharth	Emory University	*Micafungin and Amphotericin B synergy against Candida auris*	8	1
Jo	Jinhee	University of Houston	*A Multicenter Pharmacoepidemiologic Evaluation of Echinocandin Use*	33	2
Kuo	Julie	University of Houston	*Epidemiology of Carbapenem-resistant Enterobacteriaceae in a Large Academic Teaching Hospital in Houston, Texas*	57	3
Lancaster	Chris	University of Houston	*An Academic-Information Technology Partnership to Create an Infectious Diseases Translational Science Database*	9	1

Last Name	First Name	Institution	*Poster Title*	Poster #	Day of Presentation
Lu	Shuo	Baylor College of Medicine	*A Drug-Resistant β-Lactamase Variant Changes The Conformation Of Its Active-Site Proton Shuttle To Alter Substrate Specificity And Inhibitor Potency*	34	2
Marreddy	RaviKanthReddy	Texas A&M Institute of BioSciences and Technology	*Chemical Genetic Exploration of Clostridium difficile Toxin Metabolism, Toward Defining Anti-virulent Drug Targets*	45	2
Martinez Janne	Juliana	Universidad El Bosque	*Multidrug-Resistant Klebsiella Pneumoniae As The Main Colonizing Organism In Patients From Intensive Care Units (ICU) In Two High*	58	3
McNeil	Jonathon	Baylor College of Medicine	*Back to The Future: Increasing Penicillin Susceptibility among Methicillin-Susceptible*	69	3
Mehta	Heer	Rice University	*Evolutionary Leapfrogging Leads To The Failure Of A Promising Antimicrobial Strategy*	10	1
Midani	Firas	Baylor College of Medicine	*Automated Analysis Of Microbial Growth Reveals Phenotypic Diversity Of Clostridioides Difficile*	35	2
Miles	Brittany	Baylor College of Medicine	*Current Challenges and Future Directions of Antimicrobial Surfaces in the Era of COVID-19*	59	3
Moc	Courtney	MD Anderson Cancer Center	*Discordance of Ceftriaxone and Cefepime MICs in Streptococcus mitis/oralis Isolates at The*	76	3
Nanayakkara	Amila	University of Texas Southwestern	*Target Validation of Novel Antibiotic, Peptide-Conjugated Phosphorodiamidate Morpholino Oligomers*	46	2
Nguyen	April	University of Texas Health Science Center Houston	*Dynamics of Enterococcus faecalis Cardiolipin Synthase Gene Expression Reveal*	36	2
Olaitan	Abiola	Texas A&M Institute of BioSciences and Technology	*Cellular Response of C. difficile Epidemic 027 to Metronidazole-induced Oxidative Stress*	20	1

Last Name	First Name	Institution	*Poster Title*	Poster #	Day of Presentation
Panesso	Diana	University of Texas Health Science Center Houston	*Role of the LiaF in the LiaR-Mediated Response Against Daptomycin and Antimicrobial Peptides in Multidrug-Resistant Enterococcus faecalis*	21	1
Park	Jane	Baylor College of Medicine	*Discovery of Inhibitors of the KPC-2 Carbapenemase Using a Focused DNA-Encoded Library*	47	2
Price	Paul "Skip"	Eastern Michigan University	*Using Complex Microbial Communities to Identify Microbes with Cryptic Antibiotic Potential*	22	1
Rae	Meredith	University of Texas Southwestern	*Heteroresistance and Genome Sequencing of Pseudomonas aeruginosa in Cystic Fibrosis Patients*	60	3
Revtovich	Alexey	Rice University	*Novel Immune Modulators Stimulate Caenorhabditis elegans Defense against Pathogens*	12	1
Rios	Rafael	Universidad El Bosque	*Genomic Insights of hVISA Phenotype in Latin American MRSA Clinical Isolates*	37	2
Rodriguez	Mauricio	Paratek Pharmaceuticals	*Targeted Substitution of SOC with Omadacycline for CABP is Associated with Risk Reduction of Clostridioides difficile Infection and Cost Savings in the Acute Care Setting*	13	1
Rodriguez	Mauricio	Paratek Pharmaceuticals	*Subinhibitory Concentrations of Omadacycline Inhibit Staphylococcus aureus Hemolytic Activity in Vitro*	61	3
Salazar	Keiko	Baylor College of Medicine	*Anti-Viral Resistance and Phage Counter Adaptation to Pandemic E. coli*	48	2
Sánchez	Belkys	Baylor College of Medicine	*Development of Bacteriophages with Anti-Biofilm Properties as Novel Treatment for Catheter-Associated Urinary Tract Infections*	70	3
Schwarz	Cory	Rice University	*Developing a Fusobacterium Phage Cocktail to Replace Antibiotic Feed Additives in the Feed Cattle Industry*	38	2

Last Name	First Name	Institution	*Poster Title*	Poster #	Day of Presentation
Seto	Charlie	Baylor College of Medicine	*Annotation-Agnostic Metagenomic Biomarkers of Infectious Disease Susceptibility*	49	2
Sfeir	Maroun	University of Connecticut Health Center	*Adoption of the Revised CLSI Fluoroquinolones Breakpoints for Gram-negative Bacteria*	71	3
Shropshire	William	University of Texas Health Science Center Houston	*Parallel, Endemic Dissemination of Carbapenem Resistant CG307 and CG258 Klebsiella Pneumoniae Lineages with Unique Accessory Genomes in Houston, TX*	62	3
Shropshire	William	University of Texas Health Science Center Houston	*Non-carbapenemase producing organisms with CTX-M gene amplification account for majority of invasive carbapenem-resistant Enterobacterales bacteremia in immunocompromised patient population*	72	3
Sierra-Bakhshi	Cecilia	Marshall University	*The MacAB Efflux Pump is Involved in Protecting Serratia marcescens from Aminoglycoside Antibiotics, but not from Macrolide Antibiotics*	23	1
Song	Xinhao	Rice University	*Using Synthetic Ecology to Explore the Evolution of Social Interactions among Streptomycetes*	14	1
Sun	Zhizeng	Baylor College of Medicine	*Deciphering the Determinants of KPC-2 Carbapenemase Activity and Substrate Specificity Using Random Mutagenesis and Deep Sequencing*	24	1
Supandy	Adeline	Rice University	*Combinatorial Evolution of Enterococcus faecium to Daptomycin and Fosfomycin*	39	2
Talyor	Doris	Baylor College of Medicine	*Use of a DNA-Encoded Chemical Library Approach to Identify Inhibitors for the OXA-48 Carbapenemase*	75	3
Taylor	Maggie	Baylor College of Medicine	*Epidemiology of Penicillin Allergy Labels in the Pediatric Primary Care Setting*	63	3
Terwilliger	Austen	Baylor College of Medicine	*Phage Therapy for Recurrent Urinary Tract Infection in a Transplant Recipient*	15	1

Last Name	First Name	Institution	*Poster Title*	Poster #	Day of Presentation
Tran	Truc	University of Texas Health Science Center Houston	*Heterologous Expression of liaX from Enterococcus faecium in an E. faecalis Host*	40	2
Valeeva	Lia	Kazan Federal University	*Antibacterial Activity Of Extracellular Metabolites Secreted by Model Moss Physcomitrella Patens*	64	3
Vargas Otalora	Sandra	Universidad El Bosque	*Carbapenem-Resistant Pseudomonas spp. And Acinetobacter spp. Colonization In Intensive Care Units Patients From Two Hospitals In Colombia*	16	1
Vega	Luis	University of Texas Health Science Center Houston	*Contribution of a Mobile Genetic Element to Adherence, Invasion and Pathogenesis of Emergent Antimicrobial Resistant Group A Streptococcus*	41	2
Walker	Jennifer	University of Texas Health Science Center Houston	*Characterization of the Antimicrobial Susceptibility Patterns and Virulence Mechanisms Promoting Staphylococcal Medical Device Infections*	73	3
Wang	Emily	Rice University	*Multi-omic Analysis of Host-microbiome Signaling Associated with Probiotic Efficacy in Traumatic Brain Injury*	65	3
Worley	Jay	Harvard University	*Genomic Drivers of Multi-Drug Resistant Shigella Affecting Vulnerable Patient Populations in the US and Abroad*	17	1
Yu	Pingfeng	Rice University	*Hitchhiking Behavior in Bacteriophages: Implications for Phage Therapy and Biofilm Engineering*	42	2
Yu	Zhili	Baylor College of Medicine	*In situ structure of the AcrAB-TolC efflux pump at subnanometer resolution*	66	3
Zhang	Liyang	Rice University	*The oprD Deficiency Provides Fitness Advantage to Pseudomonas aeruginosa in a Caenorhabditis elegans Infection Model*	18	1

Last Name	First Name	Institution	*Poster Title*	Poster #	Day of Presentation
Zhou	Yue	Rice University	*Structural and Biochemical Studies of MurAA, an Enolpyruvate Transferase that Contributes to Cellular Fitness During Daptomycin Attack in Enterococcus faecium*	25	1
Zulk	Jacob	Baylor College of Medicine	*Evaluating Phage Therapy for the Treatment of Urinary Tract Infection*	67	3

Poster #1

An Invertebrate Model to Study Clostridioides difficile

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Background: Pre-clinical animal models to study *Clostridioides difficile* infection (CDI) generally use mice or hamster models. *Galleria mellonella* would provide an invertebrate model that is inexpensive, easy to maintain, and does not require specialized equipment which can be used as an alternative to mammalian models.

Goal: This study investigated the feasibility of using *G. mellonella* as a surrogate insect model to study CDI pathogenesis.

Methods: In the development stage, duration of pre-incubation, optimal growth temperatures, and median lethal dose (LD50) of CDI in *G. mellonella* larvae were determined to optimize the model. In the validation stage, *G. mellonella* larvae (n=10/experiment) were gavaged with 1x10^5 colony forming units (CFU) using several *C. difficile* ribotype (RT) strains (RT027, RT106, RT014/020, RT012). After inoculation, the larvae were kept at 37°C post-infection and monitored daily for 120 hours for activity and survival. Survival analysis was compared between different RT strains to assess percent survival and reproducibility of results over time. All experiments were done at least in duplicates.

Results: *G. mellonella* larvae used within 7 days of arrival provided better survival data than older larvae (6/20 (30%) vs 16/20 (80%), p=0.003). The optimal LD50 used an infection temperature was 37°C at an inoculum 1x10^5 CFU/mL. Overall, mortality was 65% in *G. mellonella* larvae given toxigenic *C. difficile* strains and 10% in controls ($p<0.001$). Mortality ranged from 31-85% between different ribotypes. Inter-day variability was less than 3.6% difference in survival for all strains with a variability range between 2-20% for each individual RT experiments. Treatment with vancomycin reduced mortality to 10% compared to 65% for untreated controls (p=0.008).

Conclusion: We have developed a pre-clinical *G. mellonella* larvae model for CDI that was reproducible and able to demonstrate the effect of antibiotic treatment on positive outcomes. This high-throughput model will be used for future pharmacology studies investigating pharmacokinetics and pharmacodynamics of antibiotics in development for CDI.

Acknowledgements: No relevant financial relationship exists.

Poster #2

Bacterial Complications in COVID-19 Patients and Trends in Rate of Positive Bacterial Cultures Before and After COVID-19 Pandemic in a Large Single Healthcare System in the Houston Metropolitan Area

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Background: Bacterial infections are often complicate viral respiratory infections. Bacterial co-infection in patients with COVID-19 has been estimated at 3.5%, with secondary bacterial infections at 14.3% in a large meta-analysis study. A multicenter study analyzing the epidemiology of bacterial co-infections in COVID-19 patients has not yet been published on data from the Greater Houston area.

Goals: The objective of this study is to describe trends of bacterial co-infections based on culture results in hospitalized and outpatient patients diagnosed with COVID-19 in 13 hospitals comprising a large health system in Houston, Texas. This study will also compare infection rates from 2018 and 2019 to rates from 2020 to determine potential changes in species or sources of infection.

Methods: This is a descriptive analysis of patients with a positive bacterial culture after diagnosis with COVID-19. Positive cultures with the same bacterial species from the same source were not counted multiple times if taken within 2 weeks of initial culture. Species (non-*Staphylococcus aureus* staphylococci) often considered blood contaminants were excluded. Cultures were classified as within 3 days of admission or after 3 days of admission using the hospitalization in which the patient tested positive for COVID-19 to define positive cultures that were likely hospital-associated.

Results: A total of 13,481 patients tested positive for COVID-19 in both outpatient and inpatient settings before 9/1/2020. There were a total of 2,246 total positive cultures in this cohort from 1,163 individual patients (8.6% total patients), 333 from blood and 768 from respiratory sources. The most common pathogen in blood cultures was *S. aureus*, followed by *Enterococcus* and *E. coli*. Respiratory cultures were dominated by *S. aureus*, *Klebsiella* species, and *Pseudomonas* species. Cultures taken after 3 days of admission were mostly from blood (16.0%) and respiratory sources (48.5%), rather than urine (18.2%). In contrast, urine cultures were more frequent within 3 days of admission (54.1%). *E. coli*, *S. aureus*, and *Klebsiella* species were common in cultures from all sources both within and after 3 days of admission. *Enterobacter* species, *Pseudomonas* species, *Serratia marcescens* and *Stenotrophomonas maltophilia* were more common in cultures taken more than 3 days after admission. The rate of positive cultures was lower in 2020 than the previous two years, except for cultures from blood (4.83 positive cultures/1,000 patient days) and respiratory sources (5.56 positive cultures/1,000 patient days).

Conclusion: There are important differences between source and species of pathogen depending on when the culture was taken during hospitalization course. Although total culture rates are lower in 2020, blood and respiratory culture rates per number of patient days did not decline compared to previous years.

Acknowledgements: This is an unfunded study.

Poster #3

Transcriptomic Alterations Associated to Heterogeneous Vancomycin-Intermediate Staphylococcus aureus (hVISA) Phenotype in Latin-American MRSA Isolates

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Background: Vancomycin (VAN) is a first-line agent in severe infections caused by MRSA, particularly in Latin-American (LA) countries where therapeutic options are limited. The development of reduced susceptibility to VAN has been associated with changes in the regulation of multiple genes resulting in alterations in the pentose phosphate pathway, nutrient transport systems and Virulence. Nevertheless, a comprehensive picture of the molecular mechanism responsible for this phenotype remains unclear.

Hypothesis/Goals: In order to understand this phenotype, we sought to determine transcriptional alterations potentially associated with the hVISA phenotype in circulating isolates in LA.

Methods: Using the RNA-Seq technique, the transcriptomic profile of four MRSA isolates exhibiting hVISA phenotype, that were recovered from bacteremic patients in Argentina, Chile, Colombia and Ecuador was determined. Further, the transcriptomes of these hVISA isolates were compared with four genetically related vancomycin susceptible (VSSA) clinical isolates, and the reference strains Mu3 (hVISA), and N315 (VSSA). A total of 2670 transcripts genes were obtained by EDGE-pro and differentially expressed genes (DEG) were identified with DESeq2 using Benjamini & Hochberg correction (p=0.05). DEG were obtained either by pairwise comparison between hVISA/VSSA and comparing expression levels to N315 and identifying common DEG on hVISA no differentially expressed on VSSA.

Results: In the pairwise comparison, we found numbers of DEG ranged between 189 to 645. Further, DEG average number varied according to the genetic lineage, with 326, 205 and 645 DEG for ST5, ST1342 and for ST8 respectively. Only, *lacD* and *sdrD* genes had common differential expression among all hVISA transcriptomes, but this was not observed in the VSSA group. However, when the analysis was restricted to the ST5 lineage, 21 and 15 DEG was identified in the pairwise and in the comparison to N315, respectively. These DEG included virulence factors such as capsule (*capE*) and genes of the type VII secretion system (T7SS) (*esaA* and *essA*), *walK*-regulated autolysin (*ssaA*), a membrane protein that controls the access of murein hydrolase to peptidoglycan (*cidA*) and a component of the regulatory system (*agrD*).

Conclusions: The differences in the transcriptomic profile of Latin-American hVISA strains suggest that evolution of decreased susceptibility to VAN is not conserved among different lineages. However, transcriptomic profiles of ST5 strains revealed more conserved pathways, involving genes related to cell envelope. Moreover, we found transcriptomic alterations in genes not previously associated to the development of VISA/hVISA phenotype (i.e. T7SS). Therefore, this work contributes to understanding the development of this complex resistance mechanism in regional strains.

Acknowledgments: This study was funded by MinCiencias (COL130874455850) and Universidad El Bosque, PCI 9510–2017.

Poster #4

Prevalence of the Cefazolin Inoculum Effect in a Prospective Cohort of Patients with MSSA Bacteremia in Texas and the Impact of Treatment with Cefazolin or Nafcillin

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Background: Cefazolin (CFZ) or an anti-staphylococcal penicillin such as nafcillin (NAF) are the mainstay of therapy for Methicillin Susceptible *Staphylococcus aureus* (MSSA) bacteremia. Observational studies have described a lower incidence of adverse events and better outcomes in patients treated with CFZ. However, the cefazolin inoculum effect (CzIE), defined as an increase in the MSSA cefazolin Minimal Inhibitory Concentration (MIC) to ≥16 mg/L when the inoculum is 10^7 CFU/mL, continues to be a concern for the treatment of invasive MSSA infections with CFZ.

Hypothesis/Goals: We sought to describe the prevalence of the CzIE in MSSA isolates from a prospective cohort of patients with bacteremia in Texas and the differences between those patients with isolates exhibiting the CzIE treated with CFZ vs NAF. We postulated that patients with CzIE positive MSSA treated with CFZ would exhibit worse outcomes compared to those treated with NAF.

Methods: We conducted a prospective observational study including adults with a diagnosis of MSSA bacteremia treated with CFZ or NAF as definitive therapy from 14 acute care hospitals in Houston, Texas between February and September 2020. Treatment was at discretion of the attending physician. Patients with a single blood culture, polymicrobial infection, receiving combined antimicrobial therapy or treatment for <72h were excluded. We determined the CFZ MICs using broth microdilution at standard (10^5 CFU/mL) and high (10^7 CFU/mL) inocula. A univariate analysis was performed to evaluate the differences between patients with the CzIE treated with CFZ vs NAF. Outcomes included in-hospital mortality, incidence of renal failure, length of stay (LOS), intensive care unit (ICU) admission, incidence of adverse events with NAF or CFZ, and persistent or recurrent bacteremia. A p-value <0.05 was considered significant.

Results: A total of 130 patients with MSSA bacteremia were included. The prevalence of the CzIE was 21.5% (n=28). Table 1 shows the demographic and clinical characteristics of patients with isolates exhibiting the CzIE compared to those without. CFZ was used in 21 (75%) patients with isolates exhibiting the CzIE. The differences between those patients treated with NAF and CFZ are shown in Table 2. In the univariate analysis, patients with high inoculum infections were more frequently treated with NAF (4.3% vs 42.9% P= 0.038). No significant differences were observed in the Charlson comorbidity index or Pitt bacteremia score between groups. ICU admission was significantly higher in patients who received NAF compared to CFZ (71% vs 19% P = 0.020). Treatment related adverse events (28.6% vs 0% P = 0.056), recurrent bacteremia (14% vs 0% P=0.25) and persistent bacteremia (42.9% vs 9.5% P= 0.085), were more frequent in patients treated with NAF vs CFZ. Inpatient mortality was similar in both groups.

Conclusions: The prevalence of the CzIE in our study was similar to that of other studies in the US. Patients with MSSA bacteremia exhibiting the CzIE who were treated with CFZ did not have worse clinical outcomes compared to those patients treated with NAF, but were also more likely to have a low inoculum infection.

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Poster #5

Genetic Context of Mercury Resistance Genes in Latin-American Staphylococcus aureus Strains

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Background: USA300-LV (Latin American variant) is the predominant MRSA clone in Colombia, that contains a genomic island designated "COMER" harboring genes for copper (Cu) and mercury (Hg) tolerance, adjacent to the SCC*mec* element. We have observed a high prevalence of Heavy Metal (HM) resistance genes (Cu and Hg) in clinical isolates of *S. aureus* from Colombia (USA300-LV and Chilean/Cordobes clones), which suggest that the environment could be driving the evolution of this pathogen in our region. HM environmental contamination is a serious threat to public health in developing countries and could also influence the selection and evolution of HM resistance genes in MRSA. Aditionally, Colombia is the 3rd country behind China and Indonesia, releasing the biggest / largest amount of Hg to the environment.

Hyphotesis/Golas: Our hypothesis is that the HM resistance genes were acquired by the USA300-LV clone providing it an advantage that might be related with the co-acquisition of antibiotic resistance genes. However, how these genes were acquired by *S. aureus* in the Latin American region and how these genes are transmitted is still unknown. Moreover, analyses of genetic context of mercury resistance genes (MRG) in other strains than USA300-LV have not been conducted. In this study, we aimed to characterize the genetic context of HM resistance genes in LA *S. aureus*.

Methods: We sequenced 6 MRSA and 2 MSSA clinical isolates harboring MRG from Colombia, Ecuador, Peru and Chile using short-read (Illumina) and long-read (ONT) sequencing platforms. Hybrid assemblies were constructed using Flye and iterative polishing with Medaka and Racon. Identification of insertion sequences, rearrangements and assessment of the genomic context was investigated using ISfinder, MAUVE, PlasmidFinder and SnapGene.

Results: Highly contiguous genome assemblies allowed us to identify the localization and genetic background of HM resistance genes. For MRSA belonging to USA300-LV (SCC*mec*IVc/E) and Brazilian (SCC*mec*III) clones, we confirmed the presence of HM resistance genes within SCC*mec*. In contrast, for the 4 MRSA belonging to Chilean/Cordobes clone (SCC*mec*I), collected from Colombia, Chile and Peru, MRG were located on ~30kbp plasmids genetically related that also contained the *blaZ* beta-lactamase and cadmium/arsenic resistance genes. In MSSA strains, we observed both plasmidic and chromosomal localizations of MRG. Interestingly, in one of the MSSA, MRG were inserted downstream of *orfX*, along with *repA*, suggesting a plasmidic origin. In all these cases, MRG were flanked by IS6 family elements.

Conclusion: The mobile elements SCC*mec*IVc y SCC*mec*III seem to facilitate the mobilization of the *mer* resistance genes. Thus, the acquisition of mercury genes in the Chilean/Cordobés-I-ST5 clone occurred through conserved plasmids. The MRG have been found along with both, antibiotic and other heavy metal resistance genes. Thus, our findings suggest that multiple environmental factors are contributing to the selection and establishment of successful MRSA lineages in our region.

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Poster #6

Impact of a Fluid COVID-19 Treatment Algorithm on Clinical Outcomes and Characterization of Disease Progression

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Background: The coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread globally throughout late 2019 to 2020. In response to evolving treatment recommendations, Houston Methodist Hospital System in the Greater Houston area established a COVID-19 Treatment Task Force in March of 2020 responsible for fluid treatment algorithm to guide clinicians treating infected patients.

Goals: Due to excellent documentation of algorithm changes implemented and an abundance of biomarkers collected, this retrospective, cohort study aims to evaluate clinical response rates to a variety of treatment regimens given to patients during the course of the COVID-19 pandemic as well as to characterize biomarkers predictive of severe disease progression and the superinfections occurring within this population.

Methods: Hospitalized, adult patients with laboratory confirmed COVID-19 disease admitted between March 12, 2020 to May 31, 2020 at Houston Methodist Hospital are eligible for inclusion in this study. The primary objective of this study seeks to evaluate the different COVID-19 treatments' effect on clinical cure rate. Clinical cure rate will be defined as an improvement in the 6-category ordinal scale score. The categories are as follows: 1) discharged alive; 2) hospitalized with no invasive ventilation and without superinfection; 3) hospitalized with no invasive ventilation and with superinfection; 4) hospitalized with invasive ventilation and without superinfection; 5) hospitalized with invasive ventilation and with superinfection; and 6) deceased. Secondary objectives include characterizing 28-day mortality, time from symptom onset to treatment initiation, disease progression relative to biomarker levels, and bacterial and fungal co-infection rate within our population.

Results: A total of 404 patients were included within the study timeframe. Based on algorithm changes throughout the study time period and most common treatment combinations, the patients were grouped into 6 different groups: 1) remdesivir only (n=56), 2) tocilizumab only (n=41), 3) plasma only (n=26), 4) remdesivir + tocilizumab (n=39), 5) remdesivir + plasma (n=12), or 6) standard of care (n=191). All groups had greater than 50% clinical cure rate at day 28 with groups 1, 3, 5, and 6 also having greater than 50% at day 14. Higher percentages of 28-day mortality were observed in tocilizumab containing groups (tocilizumab only 7/41 (17.1%); remdesivir + tocilizumab 5/39 (12.8%)). Overall study population incidence of co-infection was 14.1% (57/404), with the highest incidence in the remdesivir + tocilizumab group at 30.8%.

Conclusions: Results do not show a preference to a singular COVID-19 treatment group for the primary outcome of clinical cure rate. Higher mortality rates are observed in tocilizumab containing treatment groups. Procalcitonin shows promise as a predictive biomarker for poor clinical outcomes.

Acknowledgements: Houston Methodist Hospital System

Poster #7

Development of Novel Antifungals Against Candida Based on an Antifungal Peptide Produced by E. faecalis

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Fungal resistance to commonly used medicines is a growing public health threat. The most common cause of dangerous, bloodstream, fungal infections is *Candida* species, and there are emergent strains of *Candida* resistant to all current antifungals. To increase the probability of successfully treating *Candida* infections, novel antifungals must be developed. The basis of our project in developing a novel antifungal agent is a secreted bacterial peptide, EntV, which is produced by *Enterococcus faecalis* and restricts *C. albicans* to a non-virulent form. By targeting virulence rather than viability, the chances of developing resistance to EntV may be less than traditional antifungals. Our investigation aims to identify the minimal structural features necessary for EntV activity, generate a combinatorial peptide library using the truncated peptide as a template, conduct high-throughput screening to determine gain-of-function peptide variants, and test EntV and its variants in preclinical models to determine its effectiveness and potential usage. We hypothesize that by rationally varying specific residues in combination, we will generate more potent antifungal peptides than the template sequence through synthetic molecular evolution. We will use *C. albicans* to screen the novel antifungal peptides that we generate, as that is the causative agent behind most *Candida* infections. We expect that our discoveries will contribute to the development of novel antifungals in the fight against antimicrobial resistant fungi.

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Poster #8

Micafungin and Amphotericin B Synergy Against Candida auris

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Background: *Candida auris* is an emerging pathogen that has now been detected in all 6 inhabitable continents since it was first reported in 2009. Most *C. auris* isolates are resistant to multiple classes of antifungals. Echinocandins (micafungin) are the current recommend first-line treatment for *C. auris* infections.

Hypothesis/Goals: We hypothesized that the combination of micafungin and amphotericin B would be effective in inhibiting *C. auris* clinical isolates.

Methods: Using 10 *C. auris* isolates from the CDC/FDA AR Bank, we assayed for 1) their MICs to micafungin alone or in combination with amphotericin B using broth microdilution, 2) tested for synergy using the checkerboard assay, and 3) tested the effect of micafungin pretreatment on the efficacy of micafungin/amphotericin B combination treatment.

Results: We observed that the micafungin and amphotericin B combination caused a significant reduction (up to 64-fold) in the MIC of all the isolates compared to micafungin alone. The combination was synergistic in 8 out of the 10 isolates with FICs ≤ 0.5. Finally, the combination was effective against the isolates that were grown in the presence of micafungin.

Conclusions: We observed that the combination of micafungin and amphotericin B was effective in killing *C. auris*, including those that were already exposed to micafungin. These findings suggest that combination treatment with micafungin and amphotericin B might have clinical use against *C. auris*. These findings are significant because *C. auris*-infected patients are likely to be treated with micafungin before susceptibility data are available, and our data suggest that the combination of micafungin and amphotericin B is effective even against isolates that are already exposed to micafungin.

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Poster #9

An Academic-Information Technology Partnership to Create an Infectious Diseases Translational Science Database

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Background: Translational science is the process of turning observations in the laboratory, clinic, and community into interventions that improve human health. The coordinated effort to maintain integrated, validated laboratory and clinical data is often a rate-limiting step for research laboratories, especially for multi-site studies. Previous research shows a rate of error between 2.3 and 5.2% for basic data collection in clinical databases, up to 26.9% for more complex data points.

Goals: The purpose of this project was to create a translational science database prototype that would be responsive to the unmet needs of the translational research community.

Materials/methods: Translational scientists, IT experts, and lab technicians mapped the workflow of a high-throughput research laboratory including clinical and laboratory data. Database goals were to develop processes that would minimize data entry time, avoid redundancies, and validate data in a secure environment (HIPAA-compliant). Unique to this platform was the ability to map creation of new samples (for example, PCR products) from parent samples (biologic samples). The platform was developed in an iterative process utilizing interviews, workflow study, analysis of supporting artifacts, and mock-ups.

Results: The current prototype allows for electronic upload or manual data entry of clinical data. In a small controlled study we found the rate of error for basic data entry to be below 1% within it. Pre-populated data entry screens map laboratory work-flow with custom data entry fields produced based on laboratory results earlier in the work flow. Work-flow mapping includes microbiology, phenotypic descriptions (MIC), molecular biology (PCR), and customized experiments. Sequence data, housed separately, has data linkers stored in the database. The launch screen and data entry forms are populated based on specific criteria entered for each user.

Conclusions: The Translational Science Database allows for efficient capture of high-quality data with baseline validation enabling seamless linking of translational data for single or multi-site laboratories. Future development work will expand the number of experiments and also incorporate stored biobank information into the database.

Poster #10

Evolutionary Leapfrogging Leads to the Failure of a Promising Antimicrobial Strategy

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Background: The rise of antibiotic resistant pathogens worldwide threatens to undermine generations of biomedical progress. Using combinations of antibiotics has long been considered a promising strategy, but successes have remained more elusive than anticipated.

Hypothesis/Goals: We investigated the *in vitro* efficacy of a potent combination of ciprofloxacin and doxycycline against the live vaccine strain of the select agent, *Francisella tularensis.*

Results: We showed that despite the efficacy of the individual drugs and the clear independence of their mechanistic basis of action, the two drugs together did not delay the onset of resistance and, more worryingly, the organism achieved resistance to both drugs on the same timescale as either of the drugs individually. Following the acquisition of an initial generalist mutation that provided a modest increase in resistance to both drugs, the generalized failure of the drug combination was attributed largely to an evolutionary "leapfrogging" cascade that allowed the pathogen to sequentially adapt to each drug as if they were administered separately. We observed a clear pattern where the balance of selection pressure of each drug shifted over time, allowing the organism to leapfrog over each drug independently by creating an asymmetry in the experienced selection environment.

Conclusions: These findings suggest that spatial heterogeneities in drug concentration observed *in vivo* across tissues may provide conditions that favor such an evolutionary leapfrogging and reduce the efficacy of some promising combinatorial antimicrobial therapies.

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Poster #12

Novel Immune Modulators Stimulate Caenorhabditis elegans Defense against Pathogens

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Background: Traditionally, treatments for bacterial infections have focused on killing or preventing the growth of infectious microbes. As antimicrobial resistance becomes more ubiquitous, the feasibility of this approach is beginning to wane and attention has begun to shift toward imbalancing the host-pathogen interaction in favor of the host. This can be accomplished by inhibiting bacterial virulence factors or activating host defense.

Hypothesis: We hypothesize that modulation of *Caenorhabditis elegans* innate immune pathways using small molecules can confer resistance to multiple pathogens.

Methods: We performed a high-throughput, high-content, phenotypic, fragment-based small molecule screen to identify targets that alleviate *Pseudomonas aeruginosa*-mediated killing of *Caenorhabditis elegans*. Screen hits were placed into antimicrobial, anti-virulents, or immune modulator categories based on MIC/EC ratio (MIC, minimum inhibitory concentration, amount of the compound required to interfere with bacterial growth; EC, effective concentration, minimum amount of compound required for statistically-significant rescue), and expression of *C. elegans* host defense genes. Transcriptome profiling was performed for the five selected hits. Bioinformatic, cell biology, and genetic assays were used to identify target pathways for these molecules. Compounds' ability to rescue *C. elegans* from the Gram-positive pathogens *S. aureus* and *E. faecalis* was also tested.

Results: We identified over 20 compounds that stimulated host defense gene expression. Five of these molecules were prioritized for further characterization. Four of the five compounds showed little toxicity against mammalian cells or worms, consistent with their identification in a phenotypic, high-content screen. Each of the compounds activated several host defense pathways, but the pathways were generally dispensable for compound-mediated rescue in *C. elegans – P. aeruginosa* assay, suggesting redundancy or that the activation of one or more unknown pathways may be driving protective effects. A genetic mechanism was identified for LK56, which required the Mediator subunit MDT-15/MED15 and NHR-49/HNF4 for its function. Interestingly, LK32, LK34, LK38, and LK56 also rescued *C. elegans* from *P. aeruginosa* in an agar-based assay, which uses different virulence factors and defense mechanisms. Rescue in an agar-based assay for LK38 entirely depended upon the PMK-1/p38 MAPK pathway. Three compounds, LK32, LK34, and LK56 also conferred resistance to *Enterococcus faecalis*, and the two lattermost, LK34 and LK56, increased survival of *C. elegans* exposed to *Staphylococcus aureus* as well.

Conclusions: This study supports a growing role for MDT-15/MED15 and NHR-49/HNF4 in the innate immune response. It also paves the way for future characterization of the anti-infective activity of the molecules in higher organisms and highlights the compounds' potential utility for further investigation of immune modulation as a novel therapeutic approach.

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Poster #13

Targeted Substitution of SOC with Omadacycline for CABP is Associated with Risk Reduction of Clostridioides difficile Infection and Cost Savings in the Acute Care Setting

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Background: Real-world evidence studies indicate that ~3% of hospitalized patients with community-acquired pneumonia (CAP) develop *Clostridioides difficile* infection (CDI; Chalmers et al, *J Infect* 2016). Davis risk score (DRS)≥ 6, and treatment with high-risk antibiotics (e.g., fluroquinolones [FQ] and ceftriaxone [CTX]) are associated with increased CDI risk. Omadacycline (OMC) is indicated for the treatment of community-acquired bacterial pneumonia (CABP) and has demonstrated a low propensity to induce CDI in preclinical and clinical studies. In the phase 3 OPTIC study, 2% of CABP patients who received moxifloxacin (MOX) developed CDI vs 0% for OMC (Stets et al, *N Engl J Med* 2019); 14% of MOX patients with DRS≥ 6 developed CDI vs 0% in the OMC group.

Objective: We assessed the economic impact of substituting current CABP treatment (FQ and CTX) with OMC for hospitalized CABP patients with DRS≥ 6.

Methods: A deterministic healthcare-decision analytic model was performed. Only excess costs associated with each treatment were considered. Base-case model inputs were: yearly CAP admission in US (1 million), prevalence of CAP patients with DRS≥ 6 (10%), CDI risk for CAP patients with DRS≥ 6 with current CABP treatments, CDI costs (initial [$43.7K], recurrent [$88.8K]), and OMC cost ($345/vial). Efficacy and safety of treatments were assumed to be equal. 0% CDI risk was assumed for OMC. Costs are in USD.

Results: For CABP patients, total CDI costs were $738M, with first-episode costs of $489M plus recurrence costs of $249M. The cost of 5 days (mean hospital length of stay for CABP) of OMC was $207M. OMC use for the estimated 100,000 CABP patients with DRS≥ 6 would result in a potential cost saving of up to $531M for this patient population, assuming CDI risk of 0% with OMC. As CDI is a risk from any antibiotic use, cost savings can be achieved when OMC is used in place of high-risk antibiotics when CDI risk rates exceed 3.9%.

Conclusions: Findings suggest prioritizing omadacycline use over current CABP treatments in hospitalized CABP with a DRS≥ 6 may substantially reduce attributable CDI costs. These results can serve as a basis for stewardship interventions to reduce hospital CDI rates and associated costs.

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Poster #14

Using Synthetic Ecology to Explore the Evolution of Social Interactions among Streptomycetes

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Background

Streptomycetes are Gram positive, high GC content actinobacteria typically found in soils. They have contributed more than two-thirds of all current antibiotics and have remarkable potential for secondary metabolite biosynthesis, as indicated by a huge reservoir of cryptic biosynthetic pathways in their genomes. Moreover, in their natural soil habitat, neighboring streptomycetes have evolved to form complex interspecies interaction networks mediated mostly by a rich collection of signaling molecules and other unique secondary metabolites. Within this interaction network, various biological activities are regulated by both cooperative (promotion) and non-cooperative (inhibition) interactions. However, it remains unclear how these complex networks evolve and what are the crucial factors contributing to social network dynamics.

Goal

We aim to utilize the emulsion droplet system as a high-throughput platform to study the structure and evolution of social interactions among streptomycetes.

Method

Our study uses a microfluidics-based experimental evolution platform that allows the introduction of streptomycete strains to each other under conditions that can be used to study the evolution of new biochemical relationships. The pair-wise interaction assays in this study typically involve an engineered reporter strain and a wild streptomycete isolate serving as the interaction partner.

Result

Reporter strains have been constructed by introducing fluorescence reporter systems *S. venezuelae*. Further, we have identified several inhibitive partner strains as well as a cooperative one from a wild streptomycete library isolated from natural soil samples. Particularly, wild isolate T4-11 was selected as the inhibitive partner and experimental evolution studies using both conventional flask-transfer method and emulsion droplet-based method were performed. *S. venezuelae* mutants resistant to T4-11 spent media were successfully obtained from both studies and were subject to deep-sequencing analysis. Mutations in transmembrane transporters and histidine kinases were identified in majority of the mutants. Moreover, the spent media of T4-11 has exhibited a strong and specific inhibition effect on several Gram positive pathogens including *Bacillus subtilis* and methicillin-resistant *Staphylococcus aureus* (MRSA), suggesting the presence of molecules with potential pharmaceutical significance. On the other hand, some preliminary data have demonstrated a subtle promotion effect of wild isolate AMS-5 on the growth of *S. venezuelae*.

Conclusion

Taken together, we have demonstrated the feasibility of using emulsion droplet-based system to explore the evolution of social interactions among streptomycetes.

Acknowledgement

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Poster #15

Phage Therapy for Recurrent Urinary Tract Infection in a Transplant Recipient

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Background
Bacteriophage, or phage, are the world's most prolific bacterial killer. These viral predators represent a near-infinite supply of novel anti-bacterial medicines. As such, phage are employed as a last-resort measure for patients suffering from multidrug-resistant bacterial infections.

Hypothesis/Goals
Phage are defined by their ability to adapt and evolve alongside their bacterial targets. We sought to harness this power in rationally designing a therapeutic cocktail to treat a recurrent urinary tract infection caused by a multidrug-resistant *Escherichia coli*.

Methods
We screened our library for phages that killed a clinical isolate of an ESBL (extended spectrum beta-lactamase) *E. coli* (UCS1). The 4 most promising candidates were chosen for their virulence, mucolytic properties, and ability to reduce bacterial resistance. The resulting cocktail treated a 56 year old male liver transplant patient with complex, recurrent prostate and urinary tract infections caused by the UCS1 strain. The patient received 2 weeks of intravenous phage cocktail with concomitant ertapenem for 6 weeks. Weekly serum and urine samples were collected to track the patient's response.

Results
The patient tolerated treatment without any adverse events and symptoms improved. Neutralization of phage activity occurred with sera collected 1 week after first treatment. This was consistent with immunoassays that detected upregulation of immune stimulatory analytes. The patient developed asymptomatic recurrent bacteriuria 6 and 11 weeks following the end of phage therapy – a condition which did not require antibiotic treatment. The bacteriuria was caused by a sister strain of *E. coli* (UCS1.1) that remained susceptible to the original phage cocktail and possessed putative mutations in attachment, colicin, and secretion system elements compared to UCS1.

Conclusions
The phage cocktail was well-tolerated by the patient and appeared to clear the original infection. Mutations that appeared in the sister strain UCS1.1 post-treatment might reduce its virulence and explain the patient's toleration of the bacteriuria. Sera neutralization of phage activity and corresponding upregulation of immune stimulatory analytes are suggestive of an innate and adaptive immune response to phage therapy. This study highlights the utility of rationally-designed phage cocktails and suggests that in addition to clearance, microbial succession can produce desirable clinical outcomes.

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Poster #16

Carbapenem-Resistant Pseudomonas spp. And Acinetobacter spp. Colonization In Intensive Care Units Patients From Two Hospitals In Colombia

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Background: *Pseudomonas spp.* and *Acinetobacter spp.* resistant to carbapenems (CRP/CRA) are an important cause of hospital infections, are catalogued as a public health problem and classified by the CDC as serious and urgent threat pathogens, respectively. Importantly, their ability to persisting in clinical environments is associated with resistance to antiseptics and antibiotics.

Hypothesis/Goals: We aimed to determine the frequency of colonization by *Pseudomonas spp.* and *Acinetobacter spp.* resistant to carbapenems in patients of Intensive Care Units (ICU) in two high-complexity hospitals from Colombia.

Methods: A prospective cohort study was conducted in two high-complexity hospitals between October 2019 and March 2020. Nasal and rectal swabs were obtained from adult patients admitted to the ICU on day 0 and 7 of admission, or when they presented infection. All samples were sent to laboratory for processing. Bacterial isolates were identified using CHROMagar™ Pseudomonas and CHROMagar™ Acinetobacter and the clinical data was collected using REDCap platform from eCRF. Demographic variables, Charlson index (CCI), Pitt index (PBS), and clinical outcomes were analyzed.

Results: From 35 patients, 13 (37%) and 1 (3%) were colonized by CRP and CRA, respectively. The majority of these 14 patients were men (71%), average age of 60 years and admitted mainly for hospital transfers (86%). CCI and PBS values were 2 and 3, respectively. Most of the patients (57%) presented co-colonization of *Pseudomonas* and *Klebsiella pneumoniae* resistant to carbapenems during hospitalization in ICU and it was observed only in samples obtained on day 7. The most frequent comorbidity was COPD (71%) and 43% of the cases reported use of antibiotics, including anti-pseudomonals such as carbapenems, third and fourth generation cephalosporins and piperacillin-tazobactam. The mortality was 14%, whereas, 86% of patients were discharged home, and 14% remained in ICU.

Conclusions: Colonization by CRP/CRA is highly prevalent (40%) in patients from two ICUs in Colombia and their presence is related to an increased use of anti-pseudomonals antibiotics. *P. aeruginosa* was the main non-fermentative colonizing bacteria and its concomitant co-colonization with *Klebsiella pneumoniae* was a key feature. Given the complexity of the epidemiology of CRP/CRA infections continuous surveillance of emerging pathogens in hospitals from Colombia is critical need.

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Poster #17

Genomic Drivers of Multi-Drug Resistant Shigella Affecting Vulnerable Patient Populations in the US and Abroad

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Background: An active international outbreak of multi-drug resistant (MDR) *Shigella* infections has been identified among men who have sex with men (MSM). New macrolide-resistant strains complicate treatment, a finding confounded further by lack of validated clinical breakpoints for macrolide resistance in *Shigella*. Analysis of strains isolated in the UK and Australia has implicated pKSR100-family plasmids as drivers of resistance, but without definitive characterization of their biological properties.

Goals: Determine the genomic drivers of the MDR phenotype and epidemiologic risks for infection to inform medical, public health and clinical microbiologic approaches to counter this outbreak.

Methods: Microbiological methods identified plasmid-based drivers of phenotypic resistance. Genomic-epidemiologic analyses utilized the NCBI Pathogen Detection Isolates Browser to identify genetic drivers of AMR and demographic associations.

Results: *S. sonnei* carrying 12 plasmids was isolated from a sentinel shigellosis case in an MSM patient. Two plasmids conferred MDR phenotypes which caused challenges for patient treatment. pMHMC-004 confers azithromycin resistance and is closely related to pKSR100. Genomic-epidemiologic analyses of 2097 US *Shigella* isolates revealed high carriage rates of pMHMC-004 predominantly in US isolates from men, and not in other demographic groups. Plasmid sequences were variable in their AMR gene content.

Conclusions: Azithromycin resistant *Shigella* with pMHMC-004/pKSR100 are enriched among US shigellosis cases in men, consistent with transmission among MSM. Findings informed clinical actions to improve patient care and public health efforts across CDC, FDA, and NIH.

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Poster #18

The oprD Deficiency Provides Fitness Advantage to Pseudomonas aeruginosa in a Caenorhabditis elegans Infection Model

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Background: Antibiotic resistance is an inevitable challenge we are facing in public health. One of the strategies to avoid the outbreak of antibiotic resistance is to minimize the development of antibiotic-resistant strains by reducing the pressure to evolve drug resistance. Previous studies postulated that the acquisition of antibiotic resistance would undermine the fitness of pathogens. However, recent studies revealed that both intrinsic and acquired antibiotic resistance may enhance the fitness of *Pseudomonas aeruginosa*, one of the most common multi-drug resistant pathogen causes of nosocomial infections. OprD is an outer membrane porin that mediates the cell entry of carbapenem, amino acids, and peptides. OprD deficiency would likely contribute to carbapenem resistance, but its effect on fitness has not been studied thoroughly.

Hypothesis/Goals: We aim to better understand how OprD affects the colonization and the virulence of *P. aeruginosa*. Loss of *oprD* may confer an advantage to *P. aeruginosa* both on antibiotic resistance and fitness.

Methods: *Caenorhabditis elegans* as an infection model possesses the advantages of conserved innate immune pathways, short lifespan, easy genetic manipulation, and susceptibility to infections. *C. elegans*-*P. aeruginosa* infection models can be used to evaluate colonization and to elucidate virulence mechanisms through a worm CFU (Colony-Forming Unit) assay and worm killing assay, respectively.

Results: Here, we found that wild-type and *oprD* mutant *P. aeruginosa* exhibited similar colonization in a *C. elegans* infection assay. This was consistent with their virulence, which was comparable. Remarkably, *P. aeruginosa oprD* mutants demonstrated increased colonization compared to wild type in a *C. elegans* co-infection model. To further investigate the role of OprD in fitness, we assessed the virulence and colonization ability of clinically-isolated *oprD* mutants in *C. elegans*. The majority of clinical isolates with mutations in *oprD* displayed attenuated virulence compared to PA14 (a clinical isolate with a wild-type *oprD* allele). Virulence in these isolates correlated with the production of pyocyanin, a secreted toxin. Currently, we are assessing the clinical isolates' fitness in a co-infection assay.

Conclusions: Taken together, our results suggest that there is a trade-off between increased colonization and reduced virulence in carbapenem-resistant *P. aeruginosa*.

Acknowledgements: We thank Morgan Hakki (Oregon Health and Science University) for providing clinically-isolated *oprD* mutant *P. aeruginosa.*

Poster #19

Pyoverdine Antivirulents Synergize with Gallium Nitrate to Inhibit Pseudomonas aeruginosa

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Background

Pseudomonas aeruginosa is a Gram-negative, multidrug-resistant, nosocomial pathogen that frequently causes ventilator-associated pneumonia in intensive care units and debilitating chronic lung infections in cystic fibrosis patients. The rising prevalence of drug-resistant bacteria demands new therapeutic avenues to treat *P. aeruginosa* infections. The major siderophore pyoverdine is a key virulence factor produced by the pathogen that can be targeted for therapeutic intervention. Pyoverdine not only provides the bacterium with ferric iron, a micronutrient necessary for growth, but also regulates the production of secreted toxins. Furthermore, we recently demonstrated that pyoverdine is capable of disrupting host mitochondrial homeostasis in *C. elegans* and murine macrophages even in the absence of live pathogen. Pyoverdine production also confers resistance to gallium nitrate. Gallium nitrate has been recognized as a promising antipseudomonal agent that inhibits *P. aeruginosa* growth by functioning as a ferric iron mimetic, inhibiting bacterial ferroproteins and disrupting pathogen iron uptake. However, pyoverdine irreversibly binds the metal, preventing it from reaching cytoplasmic targets.

Hypothesis/Goals

We hypothesized that pyoverdine biosynthetic inhibitors would mitigate *P. aeruginosa* virulence while increasing bacterial susceptibility to gallium nitrate.

Results

We demonstrated that fluoropyrimidines, such as 5-fluorocytosine. inhibit pyoverdine production and rescue *C. elegans*, macrophages, and mice from *P. aeruginosa* pathogenesis. Tetracyclines, a group of antimicrobials that disrupt ribosomal function, also disrupted pyoverdine production at subinhibitory concentrations. Pyoverdine inhibitors synergized with gallium nitrate to inhibit *P. aeruginosa* growth and biofilm formation *in vitro* and virulence against *C. elegans in vivo*.

Conclusions

Combinations of gallium nitrate with pyoverdine inhibitors such as fluoropyrimidines and tetracycline were effective not only against laboratory-adapted strains of *P. aeruginosa*, but also against multidrug-resistant clinical isolates.

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Poster #20

Cellular Response of C. difficile Epidemic 027 to Metronidazole-induced Oxidative Stress

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Background: Since the 1980s, metronidazole (MTZ) has been used to treat *C. difficile* infection (CDI). MTZ is a nitroaromatic prodrug that is reduced by anaerobes to nitro-radicals that damages cells. Although MTZ resistance has been reported among clinical isolates, there is lack of genetic information on how epidemic strains responds to MTZ-induced oxidative stress. To address this question, we analyzed epidemic 027 responses to MTZ, in terms of transcriptional changes and identified gene determinants for MTZ susceptibility through transposon mutagenesis (Tn).

Methods: Tn mutagenesis was done in epidemic *C. difficile* R20291 using pRPF215 and mutants screened for MTZ susceptibility by standard MIC methods. R20291 was exposed to MTZ (2 mg/L), and MTZ plus heme (5 mg/L) and subjected to RNA-seq and results confirmed by qRT-PCR alongside historic *C. difficile* CD196.

Results: Initial screening of ~7000 Tn mutants identified two with increased susceptibility (MIC=0.5 mg/L) compared to WT (MIC=4 mg/L). In the two mutants, insertions occurred in cysteine protease (*cwp84*) and 5-nitroimidazole reductase (*nimB*), respectively. While *nimB* is thought to detoxify MTZ in other anaerobes, its role in MTZ resistance in *C. difficile* remains unexplored. R20291 exposed to MTZ displayed DNA-damage responsive genes and extensive perturbation of cellular metabolism, the addition of heme significantly led to the reduction of these oxidative stress response genes. These results suggest that heme promoted MTZ resistance. In contrast, transcriptional perturbation by MTZ in the historic MTZ-sensitive CD196 was not rescued by heme. This might suggest epidemic strains are better geared to respond to MTZ oxidative stress. KEGG analysis showed that specific phosphotransferase system (PTS), oxidative phosphorylation and peptidoglycan biosynthesis were enriched pathways in upregulated genes. Additionally, overexpression of the *nimB* gene in MTZ-sensitive CD196 resulted in the development of MTZ resistance.

Conclusion: These results point to epidemic 027 being better able to withstand MTZ-induced oxidative stress through heme and the involvement of *nimB* in the development of MTZ resistance in *C. difficile*. These have implication for explaining why epidemic 027 show resistance to MTZ that was not previously seen in historic strains.

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Poster #21

Role of the LiaF in the LiaR-Mediated Response Against Daptomycin and Antimicrobial Peptides in Multidrug-Resistant Enterococcus faecalis (Efs)

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Background: Daptomycin (DAP) is a lipopeptide antibiotic used for the treatment of vancomycin-resistant enterococcal infections. Resistance to DAP in enterococci is controlled by the *liaFSR* three-component regulatory system. LiaS is the histidine kinase, LiaR is the response regulator, while LiaF is a transmembrane protein of unknown function. Our previous reports have indicated that deletion of isoleucine in position 177 of LiaF in a laboratory strain (OG1RF) resulted in DAP tolerance and changes the membrane architecture. Here, we aim to evaluate the role of LiaF in DAP resistance.

Methods: We generated two *liaF* mutants in *E. faecalis* OG1RF (DAP-susceptible, MIC = 2 μg/ml) harboring, ***i)*** a premature stop-codon (OG1RF*liaF**$_{11}$), and *ii)* an allele coding for deletion of isoleucine in position 177 (OG1RF*liaF*Δ$_{177}$). In addition, we complemented in *trans* the OG1RF*liaF*11* mutant using the pMSMP3535 nisin inducible vector with the *liaF* wild type and the *liaF*Δ$_{177}$ alleles (mutant allele). DAP MICs were performed using E-test. In addition, broth microdilution DAP MIC were performed in presence/absence of the N-terminal of LiaX to determine if LiaF is a possible receptor for LiaX protein. We evaluated cell membrane anionic phospholipid (AP) microdomains using 10-*N*-nonyl-acridine-orange (NAO). We also assessed activation of LiaFSR by evaluating surface exposure of LiaX by ELISA. Finally, we used the bacterial adenylate cyclase two-hybrid system (BACTH) to evaluate the protein-protein interaction between LiaF-LiaS, LiaFΔ$_{177}$-LiaS and LiaF-LiaR, which was confirmed by a β-galactosidase assay.

Results: The insertion of a stop codon in *liaF* of OG1RF did not have any effect on DAP MICs, membrane architecture or a significant increase in LiaX surface exposure, compared to wild-type OG1RF. In contrast, deletion of the codon encoding isoleucine in position 177 of LiaF increased surface exposure of LiaX more than 8-fold and resulted in redistribution of anionic phospholipid microdomains away from the septum without changes in the DAP MIC. Complementation *in trans* of OG1RF*liaF*$_{*11}$ with the *liaF*Δ$_{177}$ allele (OG1RF*liaF*$_{*11}$ pMSP3535::*liaF*Δ$_{177}$), resulted in redistribution of microdomains. DAP susceptibility did not change in the OG1RF*liaF**$_{11}$ in the presence of the N-terminus of LiaX. In contrast, DAP MIC increased from 1 to 8 μg/ml in OG1RF and in the complemented strain OG1RF*liaF*$_{*11}$ (pMSP3535::*liaF*). We also observed a positive interaction between LiaF and LiaS using the BACTH system with a stronger interaction between LiaS and the LiaFΔ$_{177}$ by the β-galactosidase assay. No interaction was observed between LiaF and LiaR.

Conclusions: LiaF is likely a signal transduction protein that functions as an activator of the LiaFSR stress response and the critical regulatory domain appears to be located in the motif of four isoleucines located in the C-terminal of the protein.

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Poster #22

Using Complex Microbial Communities to Identify Microbes with Cryptic Antibiotic Potential

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Background: The discovery of antibiotics from soil bacteria is widely regarded as one of the most significant achievements in modern medicine, enabling many important medical procedures including surgery and cancer chemotherapy. However, the increase in infections caused by antibiotic-resistant organisms is jeopardizing the effectiveness of these life-saving treatments, resulting in over 2.8 million infections and 35,000 deaths each year in the United States. New classes of antibiotics are desperately needed to avert an antibiotic crisis. Natural products have always been at the forefront of antibiotic discovery but the high rate of antibiotic rediscovery using traditional axenic cultivation techniques has limited discovery efforts. However, the genomic revolution has clearly demonstrated that there is still a vast wealth of cryptic biosynthetic potential encoded in bacterial genomes.

Hypothesis/Goals: In their natural environments, bacteria encounter a diverse array of microbial partners and competitors. Natural products, including antibiotics, likely play a role in structuring these communities so it seems reasonable that their production would be limited to situations where community interactions are present. After all, many antibiotics are self-harming for their producers despite built-in resistance mechanisms and would thus require strict regulatory controls on their production. We hypothesized that we could use complex microbial communities/interactions to identify microbes capable of cryptic antimicrobial production for future studies.

Methods: Virtually all past and present antibiotic discovery methods rely on the prior production of an antimicrobial compound before the application of a target organism for testing. We devised a new antibiotic discovery scheme, dubbed the modified crowded plate technique (mCPT), that is fundamentally different because it relies on the concept that the prolonged exposure of bacteria to either bactericidal or bacteriostatic antibiotics will eventually result in death and lysis of cells (i.e., the formation of a zone of inhibition) due to the activity of autolysins in bacterial cell walls or direct killing of non-replicative cells. This suggests that the simultaneous inoculation of both soil microbes and a target organism should result in detectable zones of inhibition over time.

Results: In practice, the mCPT method involves the simultaneous inoculation of soil microbes and a target organism, resulting in very evident, although small, zones of inhibition over time. Importantly, small zones of inhibition allow us to inoculate soil microbes at higher densities for longer time periods (months), thus providing the extended physical and/or biochemical contacts needed for the induction of antimicrobial natural products from cryptic biosynthetic gene clusters and an easy means of identifying their production. Using the mCPT, we have isolated over 2600 antibiotic-producing microbes, most of which show no antimicrobial activity under traditional axenic cultivation conditions.

Conclusions: This new methodology has greatly increased our ability to identify isolates that produce antimicrobial compounds effective against multi-drug resistant Gram-negative and Gram-positive ESKAPE-TB pathogens. However, determining more controlled conditions that also result in antibiotic production remains a challenge.

Acknowledgements: We would like to thank the Tiny Earth Program and the University of Michigan Natural Products Discovery Core for their help with this project.

Poster #23

The MacAB Efflux Pump is Involved in Protecting Serratia marcescens from Aminoglycoside Antibiotics, but not from Macrolide Antibiotics

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Background: The ABC type efflux pump MacAB was first discovered in *E. coli,* and its role in antibiotic resistance in *E. coli* to macrolide antibiotics was later confirmed. Consequent studies in other Gram-negative bacteria found the MacAB pump to be involved in providing the resistance macrolides, aminoglycosides and polymyxins. However, the role of the MacAB homolog in *S. marcescens* is yet to be determined.

Hypothesis/Goals: To determine the role of MacAB efflux pump in *S. marcescens* as it relates to antibiotic resistance.

Methods: *S. marcescens ΔmacAB* mutant strains were generated by lambda red homologous recombination in nuclease deficient *S. marcescens* TT392 strain and then transduced to *S. marcescens* SM6 via ΦOT8 phage transduction. For growth in gentamicin and erythromycin WT, *ΔmacAB* mutant, and Δ*macAB* p*macAB* strains were subcultured in Mueller-Hinton (MH) broth and incubated at 35°C with shaking until reaching 0.5 MacFarland's standard. Resulting strains were inoculated into 96-well plates containing MH broth with concentrations for gentamicin (Gen) 0; 1;2 and 4 μg/ml, and for erythromycin (Ery) 0; 64; 128; and 256 μg/ml, respectively. After inoculation plates were incubated overnight at 35°C with shaking, using Biotek Synergy HTX Microplate Reader, readings were recorded at 600nm every 15 minutes. Data analysis was performed using GraphPad Prism 9.0.

Results

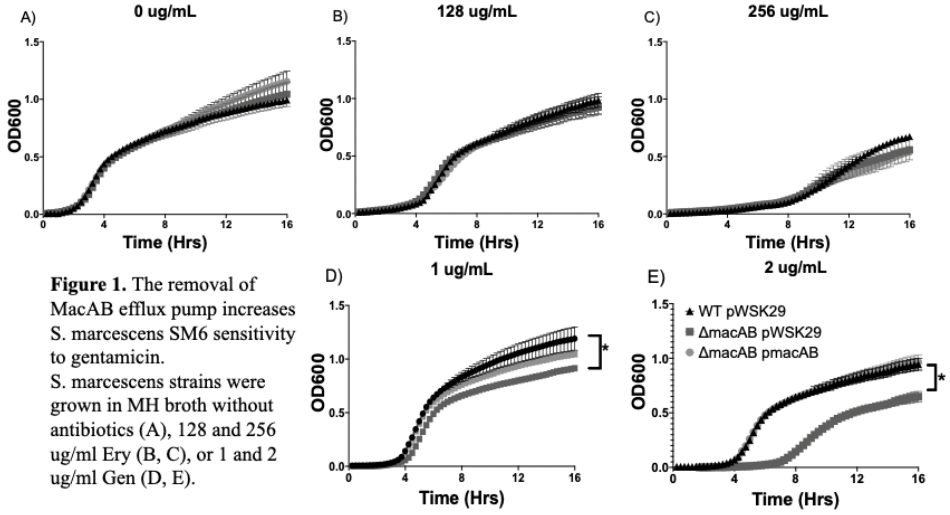

Figure 1. The removal of MacAB efflux pump increases S. marcescens SM6 sensitivity to gentamicin.
S. marcescens strains were grown in MH broth without antibiotics (A), 128 and 256 ug/ml Ery (B, C), or 1 and 2 ug/ml Gen (D, E).

Conclusion: We were able to demonstrate that removal of MacAB pump, does not alter *S. marcescens* SM6 sensitivity to erythromycin but increases sensitivity to gentamicin, that could be restored by providing *macAB* genes *in trans*. This finding makes MacAB efflux pump an attractive target for inhibition to control *S. marcescens* infections.

Poster #24

Deciphering the Determinants of KPC-2 Carbapenemase Activity and Substrate Specificity Using Random Mutagenesis and Deep Sequencing

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Background: The KPC-2 carbapenemase has broad-spectrum substrate profile that includes virtually all β-lactam antibiotics. X-ray crystal structures of KPC-2 enzyme in complex with hydrolyzed substrates indicates the important role of residues Lys234, Thr235 and Thr237 in hydrolyzing β-lactam substrates.

Hypothesis/Goals: To decipher the functional role of residues Lys234, Thr235 and Thr237 of KPC-2 enzyme in hydrolyzing penicillin, cephalosporin and carbapenem antibiotics.

Methods: Single codon randomization libraries for residue positions Lys234, Thr235 and Thr237 were constructed and selected for supporting *E. coli* cell growth in the presence of representative penicillin (ampicillin), cephalosporin (cefotaxime and ceftazidime) or carbapenem (imipenem) antibiotics. The antibiotic-resistant clones were identified by next-generation sequencing. The sequencing results were validated by determining the β-lactam hydrolysis activity of KPC-2 mutants. X-ray crystallography was utilized to determine the structure of KPC-2 mutants that displayed altered substrate specificity.

Results: Deep sequencing of β-lactam antibiotic resistant clones revealed the conservation of amino acid types in the antibiotic-selected libraries, which indicates that residue Lys234 is required for KPC-2 mediated hydrolysis of all tested β-lactam antibiotics. In contrast, residues Thr235 and Thr237 are required for the enzyme to hydrolyze cefotaxime and ceftazidime but not ampicillin or imipenem. The sequencing results were validated by the finding that K234R substitution decreased hydrolysis of all tested antibiotics while the T235S/T237S substitutions decreased cefotaxime and ceftazidime hydrolysis activity but increased ampicillin and imipenem hydrolysis activity of the KPC-2 enzyme. Determination of the X-ray crystal structure of KPC-2 T235S/T237S in complex with hydrolyzed ampicillin showed that hydrolyzed ampicillin forms very few interactions with the active site residues of the T235S/T237S mutant. Therefore, the conformation of hydrolyzed ampicillin in KPC-2 T235S/T237S might represent that for product release, which can be facilitated by the alternative conformation of the loop of Thr215 and Thr216 observed in KPC-2 T235S/T237S with or without hydrolyzed ampicillin.

Conclusions: The results show that Lys234 is an essential residue for the hydrolysis of all β-lactam antibiotics while residues Thr235 and Thr237 are important determinants of the substrate specificity of the KPC-2 enzyme. Further, the amino acid sequence requirements at residues Thr235 and Thr237 for cephalosporin hydrolysis are more stringent than those for hydrolysis of penicillins and carbapenems. Therefore, amino acid substitutions at residues Thr235 and Thr237 reduce cephalosporin hydrolysis but can enhance the hydrolysis of penicillins and carbapenems. Taken together, the results indicate that inhibitors targeting the pocket defined by residues Lys234-Thr235-Thr237 would be highly potent in blocking the activity of the KPC-2 carbapenemase.

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Poster #25

Structural and Biochemical Studies of MurAA, an Enolpyruvate Transferase that Contributes to Cellular Fitness During Daptomycin Attack in Enterococcus faecium

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Background: Vancomycin-resistant enterococci (VRE) have become a substantial concern due to its resistance to an increasingly wide range of antibiotics. Daptomycin (DAP) is an antibiotic of last resort for many patients with VRE infections. Prolonging the efficacy of DAP would be of substantial value.

Previous studies from our lab using experimental evolution to identify adaptive strategies to DAP resistance in *Enterococcus faecium* revealed that mutations in *murAA* can provide an important alternative evolutionary trajectory to resistance when the LiaFSR stress response system is inhibited. MurAA is an enolpyruvate transferase that catalyzes the first committed step of peptidoglycan synthesis (PG), transferring enolpyruvate from phosphoenolpyruvate to UDP-N-acetylglucosamine (UNAG). MurAA shows poor homology with mammalian homologs, making it a potentially excellent target for drug discovery. We are also interested in the biochemical pathways that link cell wall biosynthesis to lipid metabolism during antibiotic attack. In *Bacillus subtilis*, MurAA and the last enzyme of the PG synthesis pathway the peripheral membrane protein MurG co-localize to the division septa during exponential growth. DAP attack delocalizes MurG leading to a loss of cellular fitness. We have determined the structure of *E. faecium* MurAA and identified a clear protein-protein interaction with MurG. An adaptive mutation in MurAA identified in our experimental evolution studies markedly increased the MurAA affinity to MurG. We hypothesize that strengthening the MurAA-MurG interaction may help re-localize the PG synthesis enzymes to the division septa restoring fitness.

Hypothesis: Adaptive mutant $MurAA^{A149E}$ stabilizes the MurAA-MurG complex leading to correct localization and thereby partially restores cell wall synthesis during DAP attack leading to increased fitness.

Methods: X-ray crystallography was used to solve the structure of MurAA. The MurAA-MurG interaction was quantitated by microscale thermophoresis and dot blot assays. The localization of MurAA-MurG is being investigated by immunofluorescence microscopy.

Results and Conclusions: We solved the structure of $MurAA^{WT}$ in complex with fosfomycin, and the substrate, UNAG, in space group P1 (diffraction limit ~1.65Å). UNAG binding closes the active site loop (Ala114-Ile125) and the active site Cys119 is occupied by fosfomycin. The catalytic activity of $MurAA^{A149E}$ is only slightly reduced compared to $MurAA^{WT}$. The Kd value of MurG-$MurAA^{A149E}$ is 1.72 ± 0.13uM, while MurG-$MurAA^{WT}$ is 27.68 ± 4.00uM thus stabilizing the MurAA-MurG complex 16-fold.

Acknowledgements: Funding Sources: National Institute of Allergy and Diseases (R01 A1080714)

Poster #26

Learner Needs Assessment
Antimicrobial Stewardship Knowledge of Pediatric Residents in Ontario

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Background
The impact of antimicrobial resistance (AMR) on human health by compromising the ability to combat infectious diseases is recognized by the World Health Organization as a major health problem (WHO, 2015). The goal of the WHO global action plan is to tackle this problem and to achieve this goal through five strategic objectives, three of which address the awareness of those who prescribe or use antimicrobial agents (WHO, 2015).

Antimicrobial stewardship (AMS) programs have been shown to improve the quality of antimicrobial use in health institutes and decrease antimicrobial resistance (Schuts et al., 2016). Besides, AMS is addressed through collaborative efforts and actions across various continents (McPherson et al., 2018). The main element recommended in these action plans is educating health professionals to promote a culture of AMS (PHAC, 2017).

However, despite the availability of action plans, the rate of unnecessary antimicrobial prescriptions is still high (PHAC, 2017). Consequently, improving health professionals' knowledge about AMS has been recommended (PHAC, 2017).

Hypothesis/Goals
The purpose of this paper is to outline the learner needs assessment of the pediatrics residents' knowledge of AMS and examine the results for the future development of an educational program.

Methods
Three assessment strategies were chosen as sources of data: reflection and literature review as well as clinical guidelines, competencies, and practice standards.

Results
Reflection. It was noticed during morning rounds that the choice of antibiotics started for newly admitted children was not based on scientific grounds.

Literature review. Two medical databases: Medline (Ovid) and PsycINFO were chosen to search in the medical literature, and ERIC (Education Resources Information Center) was searched for any educational program in AMS for pediatric residents. All searches were limited by language (English), publication type (original articles and reviews), and year of publication (publications after 2000). Yet, no needs assessment of pediatric residents' knowledge and skills in AMS was found. Related associations and societies' websites, and government publications were searched for curriculum inventories or reports with no results.

Clinical guidelines, competencies, and practice standards. Both the CanMED standards of RCPSC and the ACGME program requirements for graduate medical education in pediatrics give general competencies of a pediatrician and do not include competencies specifically related to AMS (RCPSC, 2018).

Conclusion
The problem of antibiotic resistance was discussed in many forums to explore the scope of the problem. The main conclusion was how to transfer the research results to the practical ground of the health care systems (MOHLTC, 2013).

Learning needs assessment of antimicrobial stewardship program for pediatric residents is the first step in developing the program. This review shows the need for the development of an AMS educational program that will support pediatric residents in getting the knowledge, skills, and attitudes in AMS and will address the problem of antimicrobial resistance in the related context.

Poster #27

Successful Gut Decolonization of Extended-Spectrum β-Lactamase Producing Klebsiella pneumoniae Using Oral Lyophilized Fecal Microbiota Transplant (FMT) In a Woman with Recurrent Urinary Tract Infections

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Background: In patients with anatomic disruption or long-term indwelling catheters of the urinary tract, recurrent infections can be a problematic complication due to exposure to multiple antibiotics that can lead to the acquisition of resistant organisms. Restoration of a healthy gut microbiota may help such patients develop colonization resistance and eliminate multi-drug resistant organisms. We report the successful use of an oral FMT to decolonize an extended spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* (*Kpn*) from a woman with an ileal conduit with urostomy and recurrent urinary tract infections (UTI).

Methods: FMT was performed using PRIM-DJ2727, an oral encapsulated lyophilized stool product under investigation for treatment of *Clostridioides difficile* infection. Three doses of PRIM-DJ2727 (60 g total fecal matter, before lyophilization) were given weekly under an Expanded Access Investigational New Drug Application protocol approved by the US Food and Drug Administration and the local Institutional Review Board. Urine and stool samples were collected prior to treatment, 1 week after the final FMT dose, at transplant day +70, and transplant day +180. Samples underwent nucleic acid extraction using the Qiagen DNeasy PowerSoli Kit and 16S rRNA sequencing on an Illumina MiSeq. Community composition and α-diversity metrics were assessed in R version 3.6.1 using the Phyloseq package.

Results: A 50 year old woman with von Willebrand disease presented with recurrent UTIs after complications from a hysterectomy decades prior. She had an ileal conduit with urostomy, and for the prior 2 years had been colonized with an ESBL *Kpn* with recurrent episodes of pyelonephritis. In the preceding 6 months, she had 5 symptomatic UTIs. FMT was given 1 week after stopping antibiotics from the most recent UTI. In the 6 months subsequent to the FMT, she developed two symptomatic UTIs, with cultures positive for *Achromobacter* (*Axyl*), *Stenotrophomonas* (*Steno*), and *Enterococcus spp*. *Axyl* and *Steno* were not identified in the FMT product. Stool α-diversity increased after the transplant and recovered by 6 months despite oral fluoroquinolone therapy. Interestingly, there was an inverse relationship between stool and urine α-diversity. ESBL *Kpn* was not recovered subsequent to the FMT.

Conclusion: Oral FMT was used to successfully decolonize a woman with recurrent UTIs due to ESBL *Kpn*.

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Poster #28

Population Genomics Reveals Distinct Temporal Association with the Emergence of ST1 Serotype V Group B Streptococcus and Macrolide Resistance

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Background: Group B *Streptococcus* (GBS) was first recognized as a cause of infant disease in the 1970s. However, maternal intrapartum antibiotic prophylaxis (IAP) using β-lactams beginning in the mid-1990s has markedly reduced infant invasive GBS disease. GBS also causes invasive disease in adults and currently more than 90% of invasive GBS disease in the US occurs in adults, typically those with underlying medical conditions. Concomitant with the rise in adult invasive disease and introduction of IAP, GBS resistance to second line antibiotics (e.g. macrolides, clindamycin) has increased since the mid-1990s. However, little is known regarding the genetic origins and evolution of antimicrobial resistance (AMR) in GBS.

Hypothesis/Goals: Capsular polysaccharide type V (CPS V) GBS is one of the most common types to cause adult invasive disease and also is frequently resistant to second-line antibiotics. Previously, we reported that sequence type 1 (ST1) accounted for >90% of invasive CPS V GBS strains from adults. We hypothesized that ST1 serotype V GBS possessed distinct features (e.g. gene content) contributing to emergence in adult invasive disease and AMR.

Methods: A total of 59 CPS V GBS strains derived from invasive disease (1970-1992) were obtained from local (Houston, TX) surveillance. Whole genome sequencing was performed using an Illumina MiSeq instrument (300-bp, paired-end) and complete assemblies obtained for a subset using Oxford Nanopore Technologies GridION long-read sequences. Phylogenetic analyses were performed using publicly available sequences on an additional 948 CPS V strains.

Results: CPS V GBS strains isolated prior to 1992 were significantly more likely to be non-ST1 (12/32, 37.5%) compared to strains from 1992 or beyond (1/27, 3.7%; $P = 0.002$). Consistent with previous reports, nearly all isolates (53/59, 89.8%) showed resistance to tetracycline primarily attributable to the presence of *tet(M)*. However, only a single isolate prior to 1992 was found to have resistance to erythromycin (1/32, 3.1%) compared to 7 in 1992 or later (7/27, 25.9%). Moreover, the overall rate of macrolide resistance due to *erm(A)* and *erm(B)* (8/59, 13.6%) in the studied strains was significantly lower than contemporary CPS V GBS (2015-2017). Phylogenetic analysis of all GBS strains (n=1007) demonstrated distinct ST1 subpopulations strongly associated with high frequency macrolide resistance predicted to have emerged in the 1980s. Complete genome assemblies of AMR GBS strains revealed that variation in mobile genetic elements (Tn*916*-like) at a single chromosomal locus was responsible for macrolide/lincosamide resistance in the ST1 population.

Conclusions: Our findings imply that high frequency macrolide resistance coincided with ST1 emergence in adult invasive disease. That rates of AMR in ST1 GBS increased over time suggests potential enhanced fitness in strains harboring resistance elements. Further investigation of AMR contribution to and genetic features in ST1 pathogenesis may further elucidate mechanisms of GBS emergence in humans.

Poster #29

The Cefazolin Inoculum Effect in Staphylococcus aureus Bacteremia is Associated with Poor Clinical Outcomes: Results from a Prospective Latin American Bacteremia Study

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Background: The Cefazolin Inoculum Effect (CzIE) in methicillin susceptible *Staphylococcus aureus* (MSSA) is defined as an increase in the cefazolin Minimal Inhibitory Concentration (MIC) to ≥16 mg/L when the inoculum is 10^7 CFU/mL. Observational studies have described worse outcomes in patients with MSSA infections exhibiting the CzIE.

Hypothesis/Goals: We sought to describe the clinical outcomes in patients with MSSA bacteremia in a Latin American cohort. We postulated that patients with positive CzIE MSSA bacteremia had worse clinical outcomes compared to those with negative CzIE.

Methods: We conducted a prospective observational study including adults with a diagnosis of *S. aureus* bacteremia from 9 Latin American countries from January 2011 to July 2014. Patients with a survival of <48h or with polymicrobial infection were excluded. Demographic and clinical data were collected from the medical records. Cefazolin MICs were determined using broth microdilution at standard (10^5 CFU/mL) and high (10^7 CFU/mL) inocula and read at 24h. A univariate analysis was performed to evaluate the differences between the demographic, clinical characteristics, and outcomes in patients with the CzIE vs those lacking the CzIE. We performed a multivariate regression analysis to calculate the risk ratio (RR) of adverse outcomes in patients with the CzIE adjusting for age, sex and Charlson comorbidity index (CCI). Outcomes included 7-day mortality, incidence of renal failure, severity of sepsis and intensive care unit (ICU) admission. A p-value <0.05 was considered significant and the 95% confidence intervals (CI) are presented for the RRs.

Results: A total of 915 patients with *S. aureus* bacteremia were included and 506 (55.3%) were infected with MSSA. Of those patients, we were able to test 466 isolates for the CzIE. A total of 188 MSSA (40.3%) exhibited the CzIE. The median age of patients infected with isolates exhibiting the CzIE group was 61 years vs 57.7 years in those without the CzIE. Most patients were male in both groups. Table 1 shows the demographic and clinical characteristics of both groups. In the univariate analysis the median CCI was significantly higher in the CzIE group compared to the group without the CzIE (3.8 vs. 3.3 P= 0.031), while the Pitt bacteremia score was similar (median 1.6 vs. 1.5 P= 0.36). Patients in the positive CzIE group presented more frequently with peripheral vascular disease (15.4% vs. 7.9% P= 0.017) and renal failure during admission (29.9% vs 21.5% P= 0.04). In the multivariate analysis no increased risk for 7-day mortality was observed in the positive CzIE group (RR= 0.90 95% CI 0.52 – 1.57). However, patients in the CzIE group had a trend towards increased risk of renal failure (RR: 1.32 95% CI, 0.97 – 1.80), ICU

admission (RR: 1.26 95% CI, 0.97-1.64) and severe sepsis or septic shock (RR: 1.20 95% CI, 0.85-1.70) compared to the group lacking the CzIE.

Conclusions: We found that in patients with MSSA bacteremia exhibiting the CzIE there was a trend towards bad outcomes. There is increasing evidence that the CzIE plays a crucial role in clinical outcomes of patients with MSSA infections.

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Poster #30

Impact of Bicarbonate on PBP2a Production, Maturation and Functionality in Selected Methicillin-Resistant Staphylococcus aureus (MRSA) Strains

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Background: A subset of methicillin-resistant *Staphylococcus aureus* (MRSA) strains display susceptibility to the β-lactams, cefazolin and oxacillin, in the presence of $NaHCO_3$, a phenomenon termed '$NaHCO_3$-responsiveness'. This phenotype appears to be due, in part, to reduced expression of both *mecA* (which encodes the alternative penicillin-binding protein [PBP] 2a) and *blaZ* (which co-regulates PBP2a production), resulting in decreased PBP2a protein production. $NaHCO_3$ also reduces expression of two other genes important to maintenance of the MRSA phenotype, *sarA* and *sigB.*

Hypothesis/Goals: We further investigated the impact of $NaHCO_3$ in $NaHCO_3$-responsive vs. $NaHCO_3$-nonresponsive MRSA strains on: **i**) cell membrane PBP2a protein levels; **ii**) expression of the PBP2a chaperone system, VraSR-PrsA, which is required for proper PBP2a maturation; **iii**) expression of *pbp4*, a PBP required for highly cross-linking peptidoglycan in certain MRSA strains; and **iv**) expression of two key components associated with formation of functional membrane microdomains (FMMs), the scaffolding required for PBP2a anchoring within the membrane (flotillin + the carotenoid, staphyloxanthin).

Methods: Four $NaHCO_3$-responsive and four $NaHCO_3$-nonresponsive MRSA strains were used. Cells were grown in cation-adjusted Mueller-Hinton Broth (CA-MHB) + 100 mM Tris +/- 44 mM $NaHCO_3$. qRT-PCR and Western blotting were used to determine the impact of $NaHCO_3$ on gene expression (*vraSR*, *prsA*, *pbp4*, *floA*) and protein expression (PBP2a, PrsA), respectively. Carotenoids were quantified spectrophotometrically.

Results: Selectively in $NaHCO_3$-responsive strains, $NaHCO_3$ repressed: **i**) both *prsA* and *vraS* expression; **ii**) membrane PBP2a and PrsA levels; and **iii**) expression of *pbp4*. In contrast, $NaHCO_3$ repressed carotenoid production and increased expression of *floA* in both $NaHCO_3$-responsive and $NaHCO_3$-nonresponsive MRSA strains.

Conclusions: In addition to the impact of $NaHCO_3$ on *mecA/blaZ* expression, $NaHCO_3$ also impacts PBP2a and PrsA protein levels within the membrane in $NaHCO_3$-responsive MRSA strains, likely decreasing the overall functionality of the PBP2a protein. $NaHCO_3$ also selectively repressed expression of *pbp4* in $NaHCO_3$-responsive MRSA, indicating these strains may have less highly cross-linked peptidoglycan in the presence of $NaHCO_3$. Finally, $NaHCO_3$ decreased membrane carotenoid content, while increasing expression of the gene that encodes flotillin in all strains tested; this suggested a potential 'proportionality disequilibrium' in two factors required for proper PBP2a functionality within FMMs. Overall, these data indicate that $NaHCO_3$ exerts a cadre of physiologic impacts on MRSA related to PBP2a functionality that likely interplay to generate the '$NaHCO_3$-responsive:β-lactam-susceptible' phenotype in selected MRSA.

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Poster #32

Increased Mortality with Polymyxins Compared to Ceftolozane/Tazobactam in Carbapenem-Resistant Pseudomonas aeruginosa Infections

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Background: Patients with carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) have limited treatment options, prolonged hospitalizations and high mortality. Few studies have evaluated differences in clinical outcome based on treatment.

Hypothesis: Patients treated with polymyxins have a higher mortality compared to those treated with ceftolozane/tazobactam (C/T).

Methods: The Georgia Emerging Infections Program performs active population- and laboratory-based surveillance for CRPA collected from sterile sites, urine, respiratory tract and wounds in Atlanta, GA. We retrospectively reviewed charts of adults hospitalized within 1 week of CRPA culture from 8/2016–7/2018. We compared initial treatment with polymyxins (polymyxin B or colistin) to C/T using χ^2 or Fisher exact test for categorical variables and Mann-Whitney U test for continuous variables. Multivariable logistic regression estimated the impact of treatment on 30-day mortality.

Results: 34 CRPA infections were initially treated with polymyxin and 44 with C/T (5 patients had 2 unique CRPA infections). Patients receiving polymyxin were more likely to require dialysis at baseline (35% vs. 14%, p=0.02) and have CRPA isolated from a respiratory source (59% vs. 34%, p=0.05) (Table 1). 12 (35%) patients treated with polymyxin and 6 (14%) treated with C/T died within 30 days (p=0.02). In a multivariable analysis including dialysis at baseline, sterile site infection, and recent ICU admission, patients receiving polymyxin had an increased 30-day mortality over those receiving C/T (aOR 3.9, 95% CI 1.1–13.9).

Conclusions: These findings support the recent guidance favoring C/T over polymyxins for CRPA infections.

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Poster #33

A Multicenter Pharmacoepidemiologic Evaluation of Echinocandin Use

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Background: Invasive candidiasis (IC) carries a large economic burden on the US healthcare system; candidemia is reported to have attributable cost of ~$40,000 per patient. The spectrum of IC can range from minimally symptomatic candidemia to fulminant sepsis. Although *Candida albicans* continues to be the most prevalent *Candida* in IC, both drug-resistant *Candida* spp. and *Candida auris* have emerged and been designated by the CDC as serious and urgent threats, respectively. Currently, echinocandins are recommended as empiric and/or initial therapy for many forms of IC due to their activity against most *Candida* species and favorable toxicity profile. Key challenges to managing IC involve rapid initiation of appropriate systemic antifungal therapy and appropriate de-escalation based on microbiological/susceptibility data. Real-world data on echinocandin therapy, including indication, durations of use, and appropriate de-escalation, are lacking.

Hypothesis/Goals: This study aims to better understand patterns of echinocandin use through conducting a pharmacoepidemiologic analysis of echinocandins at two large healthcare systems in Houston, Texas.

Methods: All pharmacy administration and clinical microbiologic data for patients hospitalized between 2017-19 at CHI/Baylor St. Luke's Medical Center and Memorial Hermann Hospitals were screened for echinocandin use and positive *Candida* culture result. Number of unique echinocandin orders were totaled, and monthly days of therapy (DOT) per 1,000 patient days were calculated. Investigators evaluated the proportion of echinocandin-treated patients with or without positive *Candida* cultures and the antifungal discharge disposition of the first 350 echinocandin courses.

Results: Of 1,665 echinocandin courses included in the analysis, 549 courses (33%) were initiated within 2 days of hospital admission and the average time from hospital admission to echinocandin start was 9 (± 13) days. The mean (± SD) echinocandin DOT/1,000 patients was 26 (± 5) DOT and did not change appreciably throughout the study period. The mean (± SD) age of patients who received echinocandins was 57 (± 15.7) years. Of the 1,665 patients who received echinocandins, 845 (51%) had positive *Candida* cultures. The mean length of echinocandin therapy was significantly longer for patients with positive *Candida* cultures (5.5 ± 5.9 days) compared to those without positive cultures (3.9 ± 5.0 days; $p<0.001$). Analysis of the first 350 echinocandin courses demonstrated that 93 courses (26.6%) were continued upon hospital discharge and 63 courses (18%) were de-escalated to an oral antifungal agent. For those who were discharged with echinocandin and those who were de-escalated to an oral antifungal upon discharge, intra-abdominal candidiasis (41.6% and 39.7%, respectively) was the most common indication. Pre-emptive echinocandin use (40.6%) was the common indication for inpatients who did not receive any additional antifungal therapy upon discharge.

Conclusions: The rate of echinocandin use did not change appreciably during the study period. Initiation of echinocandin therapy occurred evenly throughout the hospitalization time-period and a significant proportion were continued after discharge. Further studies evaluating potential benefits of long-acting echinocandins with an emphasis of transition of care and antifungal stewardship are warranted.

Acknowledgement: This study was funded by Cidara Therapeutics.

Poster #34

A Drug-Resistant β-Lactamase Variant Changes the Conformation of its Active-Site Proton Shuttle to Alter Substrate Specificity and Inhibitor Potency

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Background:
Lys^{234} is one of the residues present in class A β-lactamases that is under selective pressure due to antibiotic use. Located adjacent to proton shuttle residue Ser^{130}, it is suggested to play a role in proton transfer during catalysis of the antibiotics. The mechanism underpinning how substitutions in this position modulate inhibitor efficiency and substrate specificity leading to drug resistance is unclear.

Hypothesis:
The K234R substitution identified in several inhibitor-resistant β-lactamase variants is associated with decreased potency of the inhibitor clavulanic acid, which is used in combination with amoxicillin to overcome β-lactamase–mediated antibiotic resistance.

Results:
Here we show that for CTX-M-14 β-lactamase, whereas Lys^{234} is required for hydrolysis of cephalosporins such as cefotaxime, either lysine or arginine is sufficient for hydrolysis of ampicillin. Further, by determining the acylation and deacylation rates for cefotaxime hydrolysis, we show that both rates are fast, and neither is ratelimiting. The K234R substitution causes a 1500-fold decrease in the cefotaxime acylation rate but a 5-fold increase in k_{cat} for ampicillin, suggesting that the K234R enzyme is a good penicillinase but a poor cephalosporinase due to slow acylation. Structural results suggest that the slow acylation by the K234R enzyme is due to a conformational change in Ser^{130}, and this change also leads to decreased inhibition potency of clavulanic acid.

Conclusions:
Because other inhibitor resistance mutations also act through changes at Ser^{130} and such changes drastically reduce cephalosporin but not penicillin hydrolysis, we suggest that clavulanic acid paired with an oxyimino-cephalosporin rather than penicillin would impede the evolution of resistance.

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Poster #35

Automated Analysis of Microbial Growth Reveals Phenotypic Diversity of Clostridioides Difficile

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Background: *Clostridioides difficile* is a gram-positive spore-forming pathogen that recently has become the most common nosocomial infection in the developed world. *C. difficile* is a genetically diverse species and distinct ribotypes are overrepresented in both human outbreaks and animals. Mass use of trehalose in food manufacturing coincided with the emergence of two epidemic ribotypes, which have a heightened ability to utilize this sugar as a carbon source.

Goals: We aimed to identify whether carbon substrate utilization by *C. difficile* isolates explains the distribution of ribotypes in the state of Texas and the novel emergence of ribotype 255.

Methods: We developed a framework for the rapid analysis of carbon substrate utilization with Biolog Phenotype Microarray carbon source plates and designed a new software, Analysis of Microbial Growth Assays (AMiGA), for modelling microbial growth curves. Using this integrative approach, we profiled clinical isolates collected through an active surveillance network in Texas.

Results: Clinical isolates generally clustered by ribotype based on their carbon substrate utilization. Ribotypes dominant in Texas (RT027 and RT014-020) exhibited higher area under the growth curve on carbon substrates commonly metabolized by *C. difficile*. Our analysis identified several substrates, such as leucine, melezitose, sorbitol, and trehalose, that are differentially metabolized by distinct ribotypes. It also showed that ribotype 255 grows faster than most ribotypes on several carbon substrates.

Conclusion: Ongoing work will continue to profile additional isolates and validate substrate-based fitness advantages with genomic verification, molecular characterization, and competition assays.

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Poster #36

Dynamics of Enterococcus faecalis Cardiolipin Synthase Gene Expression Reveal Compensatory Roles in Daptomycin Resistance

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Background: Daptomycin (DAP) is a lipopeptide antibiotic targeting membrane anionic phospholipids (APLs) at the division septum, and resistance (DAP-R) has been linked to mutations in genes encoding ***i***) the LiaFSR stress response system or its effector LiaX, and ***ii***) cardiolipin synthase. Activation of the *E. faecalis (Efs)* LiaFSR response is associated with DAP-R and redistribution of APL microdomains away from the septum, and cardiolipin is predicted to be a major component of these APL microdomains. *Efs* encodes two putative cardiolipin synthase genes, *cls1* and *cls2*. While changes in Cls1 are associated with DAP-R, the exact roles of each enzyme in resistance are unknown.

Hypothesis/Goals: This work aims to establish the contributions for both enzymes in the development of DAP-R by exploring differences in gene expression profiles, effects on DAP susceptibility (DAP-S), impact on APL microdomain localization, and membrane cardiolipin content.

Methods: *cls1* and *cls2* were deleted individually and in tandem from DAP-S *Efs* OG117 and DAP-R *Efs* OG117Δ*liaX* (a DAP-R derivative with an activated LiaFSR response). Mutants were characterized by DAP minimum inhibitory concentration (MIC) using E-test on Mueller-Hinton II agar and localization of APL microdomains with 10-N-nonyl-acridine orange staining. Quantitative PCR (qRT-PCR) was used to study gene expression profiles of *cls1* and *cls2* in *Efs* OG117Δ*liaX* relative to *Efs* OG117 across the cell growth cycle. Membrane lipid content was analyzed using hydrophilic interaction chromatography-mass spectrometry (HILIC-MS) on membranes extracted via the Bligh and Dyer method.

Results: qRT-PCR revealed differential expression profiles of *cls1* and *cls2* associated with DAP-R: *cls1* is highly upregulated in stationary phase concurrent with a decrease in *cls2* expression. However, independent deletion of *cls1* or *cls2* in the DAP-R background resulted in no significant changes in DAP MICs and maintained non-septal APL microdomain localization. qRT-PCR showed that *cls2* expression is upregulated upon deletion of *cls1* in both the DAP-S and DAP-R background at all time points. HILIC-MS analysis indicated an overall decrease in cardiolipin levels upon individual *cls* deletion relative to the parent strain. However, when comparing membrane lipid content between *Efs* OG117Δ*liaX*Δ*cls1* and *Efs* OG117Δ*liaX*Δ*cls2,* there were no significant differences in both the level of overall cardiolipin generated and the species of cardiolipin produced, suggesting a potential redundancy in the roles of both cardiolipin synthases in the development of DAP-R. Ultimately, double deletion of both *cls* genes in the DAP-R strain lowered the DAP MIC relative to the parent strain and restored septal localization of APL microdomains.

Conclusions: Overall, Cls1 has a predominant role in the development of DAP-R in *E. faecalis*, however, we describe a novel compensatory role for Cls2 under conditions in which there is no functional Cls1 to maintain the DAP-R phenotype.

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Poster #37

Genomic Insights of hVISA Phenotype in Latin American MRSA Clinical Isolates

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Background: Heterogeneous Vancomycin Intermediate *Staphylococcus aureus* (hVISA) is a complex phenotype, characterized by susceptibility to VAN (MIC ≤ 2 μg/mL) and subpopulations exhibiting reduced susceptibility to VAN (MIC 4-8 μg/mL). hVISA infections are associated with treatment failure and persistent infections. Furthermore, the genetic mechanism for the development of hVISA phenotype remains unclear. Genetic alterations in multiple and diverse systems involved in the cell wall and carbohydrate metabolism, changes in expression levels of regulatory elements and virulence genes, have been associated to the development of hVISA phenotype.

Goals: We aimed to identify genetic adaptations associated to the development of hVISA phenotype using in a collection of 344 Latin American MRSA genomes, 39 hVISA and 305 VSSA clinical isolates.

Methods: Genomes were sequenced in Illumina platform, assembled and annotated. We performed a genome wide association study (GWAS) of k-mers with PySeer. Additionally, we did a linear discriminant analysis (LDA) over single nucleotide polymorphisms (SNPs) obtained against N315. The LDA was limited to 262 genomes from clonal complex 5 CC5, as 95% of the hVISA isolates belong to it. Relevant k-mers were selected if they had a p-value < 0.01 after Bonferroni correction. SNPs identified with more than 30% difference in proportion among groups were selected as relevant.

Results: GWAS identified 4072 significant k-mers that covered 162 genomic regions (116 in coding and 47 in non-coding regions). A total of 968 SNPs were identified in CC5, the SNPs were in 286 coding and 93 non-coding regions. Eleven coding regions were identified in both approximations. Three of them were related to virulence, three to transport systems, two related to carbohydrate metabolism, two with unknown function and one associated to tRNA synthesis. The most significant k-mers in coding regions were related to mobile elements and virulence, while the SNPs with higher difference in proportions (>50%) were associated to 11 coding regions: 3 related to amino acid metabolism, 2 to carbohydrate metabolism, and one to each of the following functional groups: nitrogen metabolism, virulence, cell wall catabolism, metal transport, a phage and a hypothetical ATP binding protein.

Conclusions: In this exploratory genomic analysis of Latin American hVISA isolates, we identified multiple and varied genetic coding regions likely associated with the phenotype. Our findings support that the genetic basis of hVISA phenotype seems to be a multi-factorial mechanism. Our findings point to the relevance of further exploration of the non-coding regions and the association with

differential gene expression data. Further, these results could provide genetics insights on the hVISA phenotype in Latin American isolates and contribute with the understanding of the metabolic pathways and cellular adaptations in this complex phenotype.

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Poster #38

Developing a Fusobacterium Phage Cocktail to Replace Antibiotic Feed Additives in the Feed Cattle Industry

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Background

The beef cattle industry is under increasing regulatory and public pressure to reduce or eliminate the use of antibiotic feed additives as they are considered to be a major factor driving the widespread proliferation of antibiotic-resistant bacteria, which cause 2 million illnesses, 23,000 deaths, and cost $55 billion annually. In-feed antibiotics provide significant economic and animal welfare benefits, including the prevention of liver abscesses in feedlot cattle. This dichotomy between public welfare and the economic sustainability has created a need to identify alternatives to in-feed antibiotics. To this end, we have sought to isolate phages as an alternative to in-feed antibiotics such as tylosin, with the goal of reducing the occurrence of liver abscesses in feedlot and other grain-fed cattle by preventing the growth of the primary etiologic agent, *Fusobacterium necrophorum*. Furthermore, as host-range breadth is a key determinant of phage efficacy in complex microbial communities, such as the rumen or gut microbiome, isolation of polyvalent *F. necrophorum* phages is of particular interest.

Hypothesis/Goals

Our primary goal is the development of predefined bacteriophage cocktails that can selectively control *Fusobacterium necrophorum* within cattle rumen.

Methods

F. varium and *F. necrophorum* strains were isolated from rumen fluid using established methods. Lytic phages were isolated from enrichments of rumen fluid amended with lactate. Temperate prophages were induced from *F. necrophorum* strains using mitomycin C. Lytic phage activity was detected via spot tests, double layer plaque assays and plate reader assays. Microbial community analysis was performed using 16S rRNA gene sequencing on the Oxford Nanopore MinION system.

Results

Where previously only one phage had been isolated with lytic activity against *F. necrophorum*, we have isolated 9 phages with lytic activity against various isolated and deposited *F. necrophorum* strains. All 9 phages displayed polyvalent activity. Twenty-six lytic phages were also isolated with activity against *F. varium*. Characterization of rumen fluid microbial communities was consistent with previous studies and demonstrated a relatively low abundance of *Fusobacterium* in cattle rumen (≤ 0.003%), with *F. varium* being the dominant species identified. Preliminary studies demonstrated different phage combinations could inhibit the growth of susceptible *F. necrophorum* and *F. varium* strains *in vitro*.

Conclusions

Further isolation and characterization of these and other *Fusobacterium* phages is ongoing, and next steps involve converting temperate phages into virulent mutants while broadening host ranges to better function as targeted antimicrobial agents for use in the complex community of the rumen.

Acknowledgements

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Poster #39

Combinatorial Evolution of Enterococcus faecium to Daptomycin and Fosfomycin

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Background/Goals. Rising resistance of vancomycin resistant enterococci (VRE) towards daptomycin (DAP), a last resort drug, has pushed combination antimicrobial therapy to the forefront of VRE treatment options. One of the antibiotics being investigated for combination with DAP is fosfomycin (FOS). FOS is a phosphonic acid antibiotic that targets MurA, the enzyme involved in the first step of bacterial cell wall synthesis. It is important to determine the efficacy of this combination and to know the mechanisms by which enterococci develop resistance to this combination to start preparing alternate treatment strategies when resistance develops eventually. The purpose of this study is to explore the mechanisms by which *E. faecium* adapts to DAP and FOS individually and in combination.

Methods. *E. faecium* HOU503, a DAP-tolerant strain with $LiaR^{W73C}$ and $LiaS^{T120A}$ mutations, was chosen as the representative *E. faecium* strain. HOU503 was evolved to DAP and FOS individually and in combination through flask transfer adaptation. Minimum inhibitory concentration (MIC) for evolved isolates was determined by agar dilution in BHI agar in triplicates. Whole genome sequencing was conducted on resistant isolates.

Results. DAP and FOS exhibit indifferent/additive activity against HOU503. HOU503 adaptation to DAP-FOS combination took longer (18 days) compared to single antibiotic adaptation (8 days). DAP-resistant isolates had mutations in *cls, gdpD, pgsA,* and CDP-AP. FOS-resistant isolates had mutations upstream of *murAB* and in *pyk*. DAP-FOS-resistant isolates had all the mutations stated previously. Additionally, adaptation to FOS placed a heavy burden on cell fitness resulting in significantly delayed growth rate.

Conclusions. DAP-FOS antibiotic combination delayed the onset of resistance by almost 10 days in an *in vitro* model. HOU503 adapted to DAP-FOS combination by acquiring a combination of mutations that conferred resistance to each drug individually. These mutations affected lipid metabolism, cell wall synthesis, and central metabolism.

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Poster #40

Heterologous Expression of liaX from Enterococcus faecium in an E. faecalis Host

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Background: Daptomycin (DAP) is a bactericidal lipopeptide antibiotic that is often used for treatment of severe enterococcal infections. LiaX is a surface-exposed protein that is an important mediator of cell envelope homeostasis in *E. faecalis* upon exposure to DAP and other antimicrobial peptides. However, the role of *E. faecium* LiaX remains unknown. Here, we aimed to gain insights into the functional role of LiaX in *E. faecium*.

Methods: Deletion of *liaX* in *E. faecium* was unsuccessful after multiple attempts. Thus, we attempted to clone *liaX* in a vector and evaluate its functionality in *E. faecalis* lacking *liaX*. We used the nisin-controlled expression vector pMSP3535 for expression of *liaX* of *E. faecalis* OG1RF ($liaX_{OG1RF}$) and *E. faecium* TX1330RF ($liaX_{TX1330RF}$). Briefly, $liaX_{OG1RF}$ and $liaX_{TX1330RF}$ were cloned downstream of pNisA of pMSP3535, independently. These plasmid constructs were used to transform electroporation-competent *E. faecalis* OG1RFΔ*liaX*. For expression, cultures of cells were induced with final concentration of 50 ng/ml of nisin. Strains were characterized by staining with 10-*N*-nonyl-acridine orange (NAO) to visualize anionic phospholipid microdomains and detection of surface-exposed LiaX by a whole-cell enzyme-linked immunosorbent assay (ELISA). Control strains for the ELISA assay included **i)**: *E. faecalis* OG1RF$liaF^{*177}$ (high surface exposure of LiaX) and OG1RFΔ*liaX* (negative control), and **ii)** *E. faecium* R494 (high surface exposure of LiaX) and TX1330RFΔ*liaRliaX* (negative control).

Results: NAO staining of OG1RFΔ*liaX* demonstrated that anionic phospholipid microdomains were redistributed away from septa. Expression of $liaX_{OG1RF}$ in pMSP3535 in *E. faecalis* OG1RFΔ*liaX* successfully restored microdomains to septal and polar regions. In contrast, expression of $liaX_{TX1330RF}$ in the same host did not show any significant changes in the fluorescence staining compared to OG1RFΔ*liaX*. Using antibody against *E. faecalis* LiaX, no surface expression of *E. faecium* $liaX_{TX1330RF}$ was detected. Similarly, no surface expression of $liaX_{TX1330RF}$ was detected using antibody against *E. faecium* LiaX.

Conclusion: Our findings suggest that expression of LiaX is enterococcal species-specific and does not appear to function in the same manner as LiaX in *E. faecalis*. Our data suggest that LiaR-mediated adaptation of the cell envelope in *E. faecium* differs from that of *E. faecalis*.

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Poster #41

Contribution of a Mobile Genetic Element to Adherence, Invasion and Pathogenesis of Emergent Antimicrobial Resistant Group A Streptococcus

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Background: Recent literature has correlated antimicrobial resistance (AMR) genes with the occurrence of virulence factors within mobile genetic elements (MGE) in bacteria responsible for human disease outbreaks. Additionally, the presence of MGE can alter global gene regulation patterns that in turn may enhance a pathogen's virulence, providing a selective advantage to the maintenance and propagation of AMR that is independent of antimicrobial selective pressure. Surveillance of group A *Streptococcus* (GAS) disease in Houston, TX identified high frequency AMR encoded in MGE among GAS strains isolated from invasive infections. Among these MGE is a 65-kb integrative conjugative element (*ICESpyM92*) conferring aminoglycoside and tetracycline resistance, shared among nearly all (>98%) type *emm92* GAS isolates exclusively obtained from invasive infections. The greater frequency of AMR relative to other circulating GAS isolates detected among *emm92* isolates and their strong association with severe disease, further suggests that *ICESpyM92* gene content, independent of AMR, contributes to disease phenotype.

Hypothesis: The presence of *ICESpyM92* enhances invasiveness of GAS by altering streptococcal global gene expression.

Methods: Historical and contemporary *emm92* isolates and isogenic mutants either *cis*-complemented with *ICESpyM92* or lacking the MGE, respectively, were compared using *in vitro* (human epithelial keratinocytes) and *in vivo* (murine) models of subcutaneous infection to assess streptococcal adherence, invasion and virulence. *In vitro* global gene expression in these strains was measured and compared using RNA-sequencing.

Results: Comparisons of representative contemporary *emm92* to historical *ICE*-negative *emm92* and isogenic *ICE*-negative and *ICE*-positive mutants in these respective backgrounds indicate that the MGE contributes to GAS behavior in the skin niche. The presence of *ICESpyM92* significantly enhances GAS capacity to adhere and invade human epithelial cells *in vitro*. Contemporary *emm92* GAS exhibit greater invasiveness in a murine model of subcutaneous infection, generating a higher infectious burden during the acute phase of infection and more widespread host tissue damage, in stark contrast to the persistent localized surface abscess formed by historical *ICE*-negative *emm92* GAS. Transcriptomic analysis indicates that the presence of *ICESpyM92* alters *in vitro* expression of GAS genes previously associated with virulence, including those encoding the arginine deiminase pathway, the mannose/fructose phosphotransferase system and the fatty acid biosynthesis pathway.

Conclusions: The presence of *ICESpyM92* alters global gene expression and influences phenotypes involved in GAS interaction with the host skin niche. Genomic and transcriptomic differences between contemporary *emm92* isolates and historical isolates lacking *ICESpyM92* suggest potential mechanisms underlying the contemporary *emm92* invasive phenotype. Understanding the influence of resistance-encoding MGE on pathogen virulence and transmissibility will assist in effectively combating the emergence of AMR pathogens.

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Poster #42

Hitchhiking Behavior in Bacteriophages: Implications for Phage Therapy and Biofilm Engineering

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Interactions between bacteriophages (phages) and biofilms remain poorly understood despite the broad implications for antibiotic resistance control and microbiome engineering. Here, we demonstrate that some phages can hitchhike on motile non-host bacteria to facilitate their infection of biofilm-dwelling bacteria. Specifically, lytic coliphage PHH01 could adsorb onto the flagella of carrier bacteria *Bacillus cereus* and thus take advantage of bacterial motility in fluid conditions. Accordingly, PHH01, in the presence of *B. cereus*, was 4.36-fold more effective in infecting antibiotic resistant *Escherichia coli* in biofilm relative to free PHH01 alone. Moreover, phage infection mitigated interspecies competition and enhanced *B. cereus* colonization; the final biofilm composition increased from 9% *B. cereus* without phages to 43% with phages. Migration tests for coliphages and carrier bacteria on the *E. coli* lawn substantiated the mutualistic relationship between phages and carrier bacteria: the conjugation of PHH01 and *B. cereus* enhanced the colonization of *B. cereus* by 6.54 fold compared to *B. cereus* alone (6.15 vs 0.94 cm^2 in 24 h) and migration of PHH01 by 5.15 fold compared to PHH01 alone (10.3 vs 2.0 mm in 24 h). Metagenomic and electron microscopic analysis revealed that phages of diverse taxonomies and different morphologies could be adsorbed by the flagella of *B. cereus*, suggesting hitchhiking on flagellated bacteria might be a widespread strategy in aquatic phage populations. Overall, our study highlights that hitchhiking behaviors in phages can facilitate phage infection of biofilm bacteria and promote success of carrier bacteria in colonizing biofilms, which may open new roads for phage therapy and biofilm engineering.

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Poster #43

Predictive Regulatory and Metabolic Network Models for Systems Analysis of Clostridioides difficile

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Background: Though *Clostridioides difficile* is among the most studied anaerobes, we know little about the systems level interplay of metabolism and regulation that underlies its ability to negotiate complex immune and commensal interactions while colonizing the human gut.

Goals: To compile publicly available resources into two models and a portal to support comprehensive systems analysis of *C. difficile*.

Methods: By compiling a compendium of 151 transcriptomes from 11 studies we have generated an **E**nvironment and **G**ene **R**egulatory **I**nfluence **N**etwork (EGRIN) model that organizes 90% of all genes in the *C. difficile* genome into 297 high quality modules based on their conditional co-regulation. We also advanced a constraints-based metabolic model of *C. difficile* to uncover the metabolic networks that coordinate *C. difficile* colonization and adaptions to changing environments with host infection.

Results: EGRIN predictions, validated with independently-generated datasets, recapitulated previously characterized *C. difficile* regulons of key transcriptional regulators, refined and extended membership of genes within regulons, and implicated new genes for sporulation, carbohydrate transport and metabolism. Findings further predict pathogen behaviors in *in vivo* colonization, and interactions with beneficial and detrimental commensals. We also discovered that 15 amino acids, diverse carbohydrates, and 10 metabolic genes are essential to support *C. difficile* growth within an intestinal environment.

Conclusions: This study illustrates how the application of the EGRIN and metabolic models to new datasets offers key insights into causal mechanistic drivers of adaptive strategies of *C. difficile*, while providing a formative tool to design future transcriptomic, ChIP-seq and metabolic studies. Finally, this work reports on the democratization of disparate data, algorithms, and models of *C. difficile* through an interactive web portal (http://networks.systemsbiology.net/cdiff-portal/) to accelerate collaborative systems analysis of host-pathogen-commensal interactions.

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Poster #44

Amino Acid Substitutions in Key Regions of BlaI and BlaR Correlate with the Cefazolin Inoculum Effect in Methicillin Susceptible Staphylococcus aureus (MSSA)

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Background: The cefazolin inoculum effect (CzIE) has been associated with adverse clinical outcomes in patients with MSSA bacteremia treated with cefazolin. The CzIE correlates with the level of production of the BlaZ β-lactamase, which is regulated by BlaR-mediated cleavage of the BlaI repressor. In BlaR, the arginine in position 293 mediates signal transduction, and the serine in the 389 along with the asparagine in the 465 position are key for sensing the presence of β-lactam antibiotics. The N-terminal region of BlaI (residues 1 to 74) binds the promoter region of the *bla* operon preventing the transcription of the *blaZ* gene. Our previous experiments have shown that the regulatory portions of the Bla operon play a key role in the CzIE phenotype.

Goals: Here, we investigated variations of the amino acid sequence in BlaR and BlaI to determine if there was an association with the phenotypic differences seen in MSSA with or without the CzIE.

Methods: A total of 98 MSSA isolates from the USA were included. Cefazolin standard (5 x 10^5 CFU/ml) and high inocula (5 x 10^7 CFU/ml) minimum inhibitory concentrations (MICs) were determined by broth microdilution. The CzIE was defined as a cefazolin MIC ≥ 16 µg/ml at high inoculum. All 98 MSSA underwent whole genome sequencing (WGS). Briefly, gDNA was extracted using the DNeasy Blood and Tissue kit (Qiagen), DNA libraries were prepared using the NexteraXT DNA kit, and sequenced on Illumina HiSeq or MiSeq. The "wild-type" amino acid sequences of BlaR and BlaI were defined using *S. aureus* ATCC29213 as reference. An allotype was defined as a unique amino acid sequence in BlaI and/or BlaR proteins compared to the sequence of ATCC29213.

Results: Among the 98 MSSA isolates, 23% displayed the CzIE. The WGS analysis revealed 17 and 6 unique allotypes of BlaR and BlaI, respectively. BlaR allotypes associated with the CzIE included BlaR-1 (20%), BlaR-2 (80%), and BlaR-4 (88%). Among these specific sequences of BlaR, substitutions in position 293, which is associated with signal transduction, were identified in allotypes BlaR-1 (R293S) and BlaR-2 (R293N). Allotypes not associated with the CzIE were identical to BlaR of ATCC29213. Allotype BlaR-4 had two substitutions in close proximity to the β-lactamase sensing domain (D388N and E466K). BlaI allotypes associated with the CzIE included BlaI-1 (80%) and BlaI-2 (88%). Both of these BlaI allotypes had amino acid substitutions in the DNA binding domain of BlaI (A2T and D21G, respectively). No other changes in the DNA binding domain of BlaI were associated with the CzIE.

Conclusions: Among 98 MSSA strains, specific amino acid substitutions in the regulatory proteins BlaR and BlaI seem to be associated with the CzIE. These amino acid substitutions are located in domains of these proteins likely to influence expression of the BlaZ β-lactamase. Further experiments are needed to confirm the functional significance of the allotypes associated with the CzIE.

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Poster #45

Chemical Genetic Exploration of Clostridium difficile Toxin Metabolism, Toward Defining Anti-Virulent Drug Targets

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Clostridioides difficile infection (CDI) is the leading cause of hospital-acquired diarrhea, resulting from antibiotic-induced dysbiosis. CDI pathogenesis relies on the biosynthesis of the toxins TcdA and TcdB. While vancomycin is the main recommended treatment, it is not narrow-spectrum and further disrupts the microbiota during therapy. This is thought to contribute to recurrent disease in >20% of patients. Herein, we addressed the urgent need for narrow-spectrum anti-virulent inhibitors that reduce onset of recurrent CDI, by blocking toxin biosynthesis.

Screening of a rationally curated phytochemical library identified a molecule (**TSI-1**) that inhibited toxin production with inhibitory concentration (IC_{50}) of ~16 μM. Interestingly, **TSI-1** did not inhibit the growth of other gut bacterial species (MIC >100 μM), suggesting it was narrow-spectrum. To understand the mode of action of **TSI-1**, we performed click-chemistry proteomics, targeted metabolomics and chemical mutagenesis with ethyl methanesulfonate (EMS). Targeted proteomics identified a key enzyme in purine metabolism, as the molecular target. Consistent with this observation, metabolomics revealed **TSI-1** caused intracellular accumulation of adenosine, but depletion of ATP and GTP. Metabolic bypass experiments, in cells exposed to **TSI-1**, showed toxin production was reinstated by supplementation with purines.**TSI-1** target interaction was confirmed biophysically by Isothermal Titration Calorimetry (ITC) and in biochemical enzyme assays. Genome analysis of five EMS mutants that were refractory to **TSI-1** revealed mutation of CodY, a global transcriptional regulator. Toxin biosynthesis in a CodY-deletion mutant was not inhibited by **TSI-1**, indicating the molecule acted through CodY activation. The discovery of **TSI-1** and accompanying mechanistic studies reveal new pathways that regulate toxin biosynthesis in *C. difficile*, which can be exploited for narrow-spectrum inhibitors.

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Poster #46

Target Validation of Novel Antibiotic, Peptide-Conjugated Phosphorodiamidate Morpholino Oligomers (PPMOs)

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Background

Development of antibiotic resistance in pathogenic bacteria is recognized as a major public health threat. Loss of effectiveness of antibiotics threatens the success of many medical interventions such as general surgery, cancer therapy, organ transplantation and prosthetic implants. Novel antibiotics have entered the market rarely, as most classes of antibiotics in use were discovered several decades back. Use of broad spectrum antibiotics has been identified as a reason for antibiotic resistance. We have reported using peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) as species-specific antibiotics. The oligomer sequences (11 base pairs) of PPMOs are designed to be complementary to specific essential genes around the Shine-Dalgarno or translation start, thus inhibiting protein translation. Here we wanted to analyze the effects of genetic mutations on PPMOs which target the *rpsJ* gene of *Pseudomonas aeruginosa* (PA).

Methods

Homologous recombination was used to generate various PA PAO1 mutants with 4, 2 or 1 base-pair mutations within the 11 base pairs of *rpsJ* gene targeted by a specific RpsJ PPMO. As a control, a PAO1 strain was generated containing wild type non-mutated targeted sequence including the same construct used to generate mutants. Homologous arms with mutations were cloned into pEX18Tc cloning vector and pRK2013 was used to transfer the cloning vector into PA. Presence of mutations in PA was confirmed by sequencing the targeted area. Minimum inhibitory concentrations (MIC) assays and colony counts were performed for mutants, control, and wild type strains for the PPMO which target *rpsJ* gene. Further MIC assays were performed using a PPMO targeted for *acpP* gene and antibiotics such as colistin, meropenem and tobramycin to evaluate response of these strains.

Results

All mutants showed nearly 8 fold higher MIC values compared to wild type PAO1 or control strain with wild type gene and the construct indicating resistance in mutants towards the RpsJ PPMO. These results were confirmed by colony counts as well. However, mutants do not show an elevated MIC values for other PPMO antibiotics compared to wild types.

Conclusion

Even a single mutation within the targeted 11-base pair region is sufficient to impact the MIC of the rpsJ PPMO in PA. These experiments confirm target specificity of one lead PA PPMO. This also illustrates one potential mechanism of resistance that could be anticipated with this antisense approach. Future works includes determining what the impact of base mismatches are in specific locations on the target gene and analyzing the specificity of other genes which can be targeted by PPMOs.

Poster #47

Discovery of Inhibitors of the KPC-2 Carbapenemase Using a Focused DNA-Encoded Library

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Background: The CDC has reported at least 2 million infections and 23,000 deaths related to antibiotic resistance every year in the US. β-lactam antibiotics represent about 65% of all antibiotic use worldwide. Bacteria have evolved resistance against these antibiotics by producing β-lactamase enzymes that inactivate the drugs. *Klebsiella pneumoniae carbapenemase-2* (KPC-2) is a plasmid-encoded β-lactamase that is found in many Gram-negative pathogens. KPC-2 can hydrolyze all clinically available β-lactam antibiotics including carbapenems, which are used as last resort treatments. KPC-2 uses a serine residue in the active site to hydrolyze the β-lactam ring through a covalent, acyl-enzyme intermediate, rendering them ineffective. β-lactamase inhibitors were developed in an effort to fight an increasing number of β-lactamases. There are several effective inhibitors for KPC-2 including avibactam, relebactam, and vaborbactam. However, there is already a KPC-2 mutant identified in a clinical isolate that can confer resistance to avibactam.

Hypothesis/Goals: Since most inhibitors share a common core of the β-lactam ring or similar derivatives, it is a growing concern that bacteria will rapidly evolve resistance to the inhibitors. The goal of this project is to discover a novel core structure for KPC-2 inhibitors to combat the evolution of resistance.

Methods: This project will discover new small molecule inhibitors of KPC-2 using DNA-encoded chemical library (DEL) technology. Unlike conventional high-throughput drug screening, DEL uses diversity-oriented synthesis coupled with DNA sequence tagging to screen a broad chemical space and rapidly identify hit molecules via next generation sequencing. While DEL screening has many advantages compared to conventional screening, we aim to improve its effectiveness by creating a β-lactamase focused library. The focused library will contain functional groups such as carboxylates and tetrazoles that are known to bind the active site of β-lactamases.

Results: In preliminary studies, we screened several DEL libraries for molecules that bind KPC-2 β-lactamase and identified several small molecule compounds as hits. Subsequent assays showed CDD-1638 was the most potent, with a K_i of 100μM. However, none of these compounds showed an improvement in the ampicillin MIC for *E. coli* expressing the KPC-2 enzyme. The β-lactamase focused library containing carboxylic acid and tetrazole as a terminal functional group is currently in production. Once the focused library is synthesized, it will be screened against KPC-2 enzyme to identify tight binders.

Conclusions: The hit compounds from the preliminary studies have low potency compared to known clinically-available inhibitors. The MIC data also suggest the compounds cannot penetrate the bacterial outer membrane. The focused library will improve the likelihood of identifying a potent binder while taking full advantage of the DEL platform.

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Poster #48

Anti-Viral Resistance and Phage Counter Adaptation to Pandemic E. coli

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Background: Extraintestinal pathogenic *E. coli* (ExPEC), often multidrug-resistant (MDR), is a leading cause of urinary and systemic infections. The crisis of emergent MDR pathogens has led some to propose bacteriophage as a therapeutic. However, bacterial resistance to phage is a crucial blockade that we must understand to overcome. While phage-resistance has been explored in laboratory settings, they are poorly understood in therapeutic contexts.

Hypothesis: We hypothesize that phage-resistant isolates of *E. coli* will develop when exposed to phage in a model of murine sepsis, but at the expense of fitness costs that will make them less viable as pathogens.

Methods: We used three *E. coli* strains of sequence type (ST)131, a currently circulating pandemic strain of *E. coli* to develop resistant isolates to ϕHP3, a phage which has been used successfully with two single-use INDs. Using both a plate and murine sepsis model of phage exposure, we developed 21 resisters from the three parental strains. We then performed whole genome sequencing and assessed these resisters' fitness in biologically relevant conditions. We also developed a novel chemostat system which we used to generate an evolved phage isolate with restored infectivity in all LPS-truncated resisters.

Results: We found ExPEC rapidly develops resistance to ϕHP3. Whole genome sequencing of the resisters revealed truncations in genes involved in LPS biosynthesis, the outer membrane transporter *ompA*, or both; implicating them as receptors for this phage. Interestingly, resisters were attenuated in blood and demonstrated decreased virulence in a murine model of *E. coli* systemic infection. Several of the animal-derived resisters were also attenuated in urine, suggesting a greater hit to fitness under infection conditions. When evolved against a resister, ϕHP3 quickly regained efficacy against the target isolate and, strikingly, all the other resisters as well.

Conclusions: Our findings suggest that although resistance of pathogenic *E. coli* to our phage is inevitable, it comes at a fitness cost in its mammalian host, and new phage variants can be readily isolated by directed evolution.

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Poster #49

Annotation-Agnostic Metagenomic Biomarkers of Infectious Disease Susceptibility

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Background
Microbiome composition impacts the ability of priority pathogens to colonize the intestine and cause infection. Taxonomic biomarkers that accurately predict disease susceptibility remain inconsistent and technology dependent.

Hypothesis
Annotation-driven bioinformatic tools are not amenable to detection of structural variants and genomic content that drives microbial function outside of database "silos", such as taxonomy, presence of known antimicrobial resistance genes, collections of pathogenicity genes within a molecular pathway. A recent study demonstrated that small structural variants in the genome of an individual microbe can have reproducible associations with host health outcomes. Similarly, pathway-centric approaches can often be decoupled from a strain of origin; and screening with both first may shift focus away from unannotated material to "microbial dark matter", including novel virulence determinants.

Methods
We complement a traditional annotation-driven approach with annotation-agnostic workflows that emphasize identification of new metagenomic content that is associated with infectious disease susceptibility and treatment outcomes; which can later be processed using traditional means.

Results
We applied annotation-agnostic clustering techniques to unitigs in fecal specimens from healthy donors and patients with *C. difficile* infection, including subjects undergoing fecal microbiota transplantation (FMT), accounting for similar metagenomic representatives across samples. We also leverage annotation-agnostic changes to the metagenome assembly graph, applying the program KOMB and K-Core decomposition of the assembly graph, hypothesizing that changes in regional graph complexity is associated with gut microbiota changes. Differentiating representative unitigs into clusters based on graph connectivity allowed us to identify contigs not only of similar content, but separation by degree of interconnectedness. Visual comparison of KOMB histograms of metagenomes before and after FMT, demonstrated KOMB's utility to identify disease-specific changes in fecal microbiome composition. Unique metagenomic signatures associated with clinical remission were then subjected to annotation-based validation.

Conclusion
We conclude that changes in graph connectivity and prevalence of unique, unannotated content can identify the presence of donor-unique content in post-FMT samples that may serve as novel database-agnostic biomarkers of clinical remission and for hypothesis-driven discovery of infectious disease mechanisms.

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Poster #50

LiaX as a Surrogate Marker of Daptomycin Susceptibility in Multidrug-Resistant Enterococcus faecium Recovered from Cancer Patients

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Background: Vancomycin-resistant *Enterococcus faecium* (VR*Efm*) are leading causes of bloodstream infections (BSI) in patients (pts) with hematological malignancies (HM). Daptomycin (DAP) is commonly used to treat VRE BSI, but DAP resistance (DAP-R) in pts with HM is increasing. Current methods to determine DAP minimum inhibitory concentrations (MICs) have poor reproducibility. DAP triggers the LiaFSR cell membrane stress response pathway, resulting in the extracellular release of the protein LiaX, a novel protein we have described that functions as a regulator of the membrane response. We postulate that detection of extracellular LiaX correlates with DAP resistance in clinical strains of VR*Efm*

Methods: We used well-characterized VR*Efm* BSI isolates as reference strains to optimize a whole-cell indirect enzyme-linked immunosorbent assay (ELISA) method for LiaX detection. We assessed limit of detection and reproducibility of the ELISA LiaX method. We then assessed 56 clinical VR*Efm* BSI isolates from pts with cancer for validation. We determined DAP MICs by broth microdilution (BMD) for all isolates. We obtained patient demographic and microbiological details by chart review.

Results: The 6 reference strains demonstrated high reproducibility with low coefficient of variation. All DAP-R reference strains had increased detection of LiaX ($p<0.0001$) compared to DAP-S reference strains. Of the 56 isolates from pts, most pts (>80%) had HM. Greater than 60% of VRE BSIs were determined to originate from the gastrointestinal tract. Two of the 56 isolates were DAP-R. The LiaX test and MIC had categorical agreement on 61% of isolates. Of the isolates with disagreement, there were 20 isolates with susceptible DAP MIC but increased LiaX detection by ELISA, and there were no isolates with resistant MIC but decreased LiaX detection by ELISA.

Conclusions: Detection of extracellular LiaX has important discrepancies with routine DAP MIC and may be a more accurate marker of the DAP-mediated cell membrane response. Further characterization of the discrepant isolates by time-kill assays and evaluation of patient clinical outcomes is warranted to fully validate the performance of LiaX ELISA.

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Poster #51

Diagnostic Performance of Rapid Test for Detection of Cefazolin Inoculum Effect (CzIE) in Methicillin-Susceptible Staphylococcus aureus Recovered from Bacteremia in Latin-American Hospitals

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Background: *Staphylococcus aureus* is a leading cause of bacteremia, with substantial morbidity and mortality. Cefazolin (Cz) is a safe and well tolerated therapeutic alternative for the management of severe MSSA infections. However, the use of Cz for the management deep-seated MSSA infections has also been associated with therapeutic failures and mortality among strains displaying the Cefazolin Inoculum Effect (CzIE). The only methodology to detect the CzIE is microbroth dilution, which is cumbersome and time-consuming, and it is currently not available in standard clinical microbiology laboratories.

Goals: To assess and validate the performance of rapid test to detect the CzIE in a collection of MSSA from Latin-America (LA).

Methods: A total of 640 MSSA bloodstream isolates collected from 9 Latin-American countries were evaluated. As controls, we included MSSA TX0117 (CzIE positive), and ATCC 29213 and ATCC 25923 strains (CzIE negative). Cefazolin MICs at standard and high bacterial inocula were used as the gold-standard method. A rapid colorimetric test based on nitrocefin to detect the staphylococcal β-lactamase (BlaZ) in bacterial supernatants after ampicillin induction was evaluated in control strains and validated in the total of MSSA collection. Whole-genome-sequencing was performed to determine the BlaZ type. Sensitivity and Specificity were calculated. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were estimated based on reported prevalences of CzIE.

Results: The CzIE was detected in 39.4% of MSSA using the gold-standard. 80% of BlaZ type A isolates and 52% of BlaZ type C MSSA exhibited the CzIE. The overall sensitivity of the rapid test was 82.5% (range of 60-100%) with a specificity of 88.4% (range of 83.1-100%), with differences in the diagnostic performance according to the country. Among the isolates from Colombia, the country which provided most of them, the test showed sensitivity and specificity of 84.9% and 91.2%, respectively. Whereas Mexico, the country with the lowest number of isolates, had a sensitivity of 100% and specificity of 83.3%. PPV and NPV ranged between (59.8 to 100%) and (81.4 to 97.6%), respectively. When the performance of rapid test was evaluated according to BlaZ type, type-A isolates showed a sensitivity and specificity of 90.6% and 84.2% respectively, whereas type-C isolates had lower performance with sensitivity of 72.3 % and specificity of 81.1%, respectively.

Conclusion: Our rapid test is a simple, economic, and convenient method to identify the CzIE in MSSA. The test showed a good diagnostic performance, and it could be potentially implemented in clinical laboratories after minor validation process.

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Poster #52

Evaluation of Antimicrobial Properties of Extracts from the Model Moss Ceratodon purpureus

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Background:
Mosses are known to produce various antimicrobial compounds. Two strains of the model moss *Ceratodon purpureus* (male R40 and female GG1) were grown and processed to test for the presence of secondary metabolites with antimicrobial activity.

Hypothesis/Goals:
The goal of this research is to detect antimicrobial secondary metabolites from *Ceratodon purpureus* that inhibit the growth of bacteria.

Methods:
The male and female strains of *Ceratodon purpureus* were grown on solid and liquid BCD-NH_4-T medium. Mosses grown on solid medium were collected and used for methanol extraction while the liquid-grown moss was processed for exudates. Samples were analyzed after 2 and 4 weeks of growth on solid or liquid media. Moss extracts and exudates were tested for antimicrobial activity by two different methods. The DDM (disk diffusion method) was used to qualitatively measure bacterial inhibition zones around the disks. The broth microdilution method was used for MIC (minimum inhibitory concentration) determination. All samples were tested in triplicates against several Gram-positive and Gram-negative bacteria.

Results:
Exudates from the male *C. purpureus* strain R40 displayed antimicrobial activity against Gram positive bacteria, as determined by both DDM and MIC methods. Two-week and 4-week old moss secreted metabolites into the growth medium, which inhibited the growth of *Staphylococcus aureus* ATCC 25923. In DDM assays, the average inhibition zone for two week-old exudates was 9.83 mm, while for four week-old extracts the zone was larger, 15mm. MIC values were 25 mg/ml for 2-week old moss exudates, and 12.5 mg/ml for 4-week old moss exudates. No activity against Gram-negative bacteria was detected for the R40 exudates, and no overall activity against any bacterial strains were detected for R40 extracts and GG1 extracts and exudates.

Conclusions:
When tested against Gram-positive and negative bacteria, the male *C. purpureus* strain R40 showed inhibitory activity against Gram-positive bacteria only, while GG1 strain showed no inhibitory activity. Our next steps include performing size exclusion chromatography and mass spectrometry analysis to identify individual exudate components with antimicrobial activities.

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Poster #53

Characterization of Antibiotic Susceptibility Patterns and Virulence Mechanisms in Staphylococcus aureus Urinary Catheter-associated Isolates

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Background: Catheter-associated urinary tract infections (CAUTIs) are the most common hospital-associated infections in the US and can results in severe morbidity and mortality. Importantly, CAUTI are caused by a broader range of pathogens than non-catheter related UTIs. Many "atypical uropathogens" that cause CAUTI remain unstudied, despite being responsible for thousands of infections each year. One of these pathogens is *Staphylococcus aureus*, which primarily causes UTI in the presence of a urinary catheter. Concerningly, recent reports indicate *S. aureus* strains causing CAUTI are predominantly methicillin resistant *S. aureus* (MRSA), making infections from these isolates even more difficult to treat. Investigating the *S. aureus* strains causing CAUTI may provide insights into the emergence of drug resistant strains and the development of better treatment strategies.

Goals: This study aims to better understand the antibiotic susceptibility patterns and pathogenic mechanisms that promote recalcitrant *S. aureus* CAUTIs.

Methods: To study the mechanisms contributing to *S. aureus* CAUTI, we consented and enrolled participants requiring chronic urinary catheterization. Serial catheters were collected during standard of care catheter exchanges every ~30 days. *S. aureus* strains were isolated from catheters. The minimum inhibitory concentration and the minimum bactericidal concentration of each isolate to antibiotics commonly used to treat CAUTI were determined. Select strains were also assessed in a mouse model of UTI, to determine whether a urinary catheter was required for infection. Additionally, the isolates were tested for urease production, an enzyme that promotes UTI in other well-studied uropathogens.

Results: A total of 9 *S. aureus* strains were isolated from 6 patients (3 females; 3 males). All the strains were isolated from patient catheters, except one that had one strain isolated from their catheter and one from their urine. Antibiotic susceptibility testing revealed most strains were susceptible to cefazolin (8/9) and gentamicin (8/8), while one strain, HUC 57, was resistant to ciprofloxacin. Additionally, mice infected with *S. aureus* isolates HUC 86, HUC 97-02, and HUC-100, that were catheterized maintained higher colony forming units in the bladder compared to infected mice without the catheter. Lastly, all the isolates tested produced urease. Interestingly, urease activity in sequential strains was increased compared to the initial isolate.

Conclusions: Together these data suggest most *S. aureus* urinary catheter-associated isolates are sensitive to ciprofloxacin and gentamicin. Additionally, mouse model data from HUC 86, HUC 97-02, and HUC-100 support our previous work indicating a urinary catheter promotes *S. aureus* UTI. Lastly, the increased urease activity detected in sequential isolates suggests urease is an important virulence factor for promoting long-term urinary tract colonization. Thus, therapies that reduce or prevent urease activity may be developed as an antibiotic-sparing therapy.

Acknowledgements: UTHealth Start-up funds, Rising Star Award

Poster #54

Molecular Changes Associated with Heterogenous Intermediate Resistance to Vancomycin (hVISA) Phenotype in Methicillin-Resistant Staphylococcus aureus (MRSA) Clinical Isolates from Latin America

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Background: Vancomycin (VAN) has been the mainstay for therapy of complicated MRSA infections. However, the emergence of *S. aureus* with intermediate susceptibility to vancomycin (VISA) and heterogeneous VISA (hVISA) has compromised the effectiveness of VAN. The prevalence rates of hVISA in Latin America ranges between 0,6% to 19%. Even though some genetic features have been associated to the development of hVISA phenotype, the genetic basis of the phenotype remains unknown.

Goal: We sought to interrogate the reported genetic changes associated with hVISA/VISA phenotypes in the genomes of hVISA isolates causing bacteremia in patients from Latin America.

Methods: We included a total of 39 genomes of MRSA isolates collected in Latin American hospitals between 2006-2014, confirmed as hVISA by Glycopeptide Resistance Detection (GRD) and Macromethod (MET) E-test. Six of them were also PAP/AUC positive. Also, we included 305 genomes of MRSA isolates classified as vancomycin susceptible S. aureus (VSSA) collected in the same surveillance studies. With a comprehensive systematic literature search we selected 54 proteins with changes related to the development of VISA/hVISA phenotype, which were interrogated in a total of 344 genomes. Significant differences of the proportions about the presence of changes in the evaluated proteins among hVISA and VSSA isolates ($p<0,05$) were determined.

Results: A total of 2.983 amino acid changes were found in 47 out of 54 proteins evaluated. Of these, 815 substitutions were significantly associated with hVISA genomes, and were present mainly in 20 proteins involved in cell wall biogenesis, membrane biosynthesis, regulatory systems and DNA/RNA synthesis. Interestingly, 18 proteins exhibited 811 amino acid changes that were exclusively present in hVISA isolates, such as DltA, MutL, RpoB and RpoC. Other unique hVISA genome changes occurred in proteins involved in virulence (MsaC), ATP processing (RecA) and other transporters (OpuD and PotD). The most statistically predominant changes in hVISA genomes included Y38H in Atl (92,1%), L14I in WalK (89,7%) and E156G in VraT (89,5%).

Conclusions: We found 47 proteins that might be implicated in the development of hVISA phenotype, primarily in cell envelope metabolism, and regulatory systems. Our findings show an approximation on how the phenotype develops in Latin American isolates. Further studies are needed to understand the pathways involved in the emergence of hVISA phenotype.

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Poster #55

Tailored Antibacterials and Innovative Laboratories for Phage (Φ) Research: A New Service Center Developing Personalized Antimicrobials for Vulnerable Patients

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Phage therapy or the use of bacteriophages—the viruses of bacteria—as a therapeutic has reemerged as a viable treatment for difficult to treat multidrug resistant infections. TAILΦR or Tailored Antibacterials and Innovative Laboratories for Phage (Φ) Research was established at Baylor College of Medicine as a service center to develop these antimicrobials to treat the most vulnerable patients. We are leveraging core resources at the Texas Medical Center while developing technologies that evolve phage to accelerate production and expedite treatment. Our focus has been on developing therapeutics against the ESKAPE Pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and Enterobacter species) which are responsible for the majority of drug-resistant infections worldwide. Wastewater from local facilities across Houston has yielded lytic phage against all the clinical isolates of ESKAPE pathogens in our library. These phages are fully characterized (i.e. sequenced, imaged, adsorption rate and burst size determined) and highly purified using CsCl purification prior to use. Through FDA approval via IND or eIND 4 patients have been treated successfully and safely. Several more patients are currently beginning to be treated using our vetted phage cocktails. Given a bank of therapeutic-quality phage and clinical isolates, we aim to create a pipeline where lytic phages are discovered, purified, and formulated within a week. Such a system has the potential to reduce production costs, expedite therapeutic formulations, and improve patient outcomes.

Poster #56

Development of the High Performance Liquid Chromatography to Detect Vancomycin in Clinical Human Fecal Samples

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Background: *Clostridium difficile* infection (CDI) is a major clinical problem in the U.S. Oral vancomycin administration is a first-line therapy to treat CDI. Vancomycin is non-absorbed in the gastrointestinal tract and thus achieves fecal concentrations hundreds times higher than the MIC value of wild-type *C. difficile* strain. However, we have identified *C. difficile* isolates that are tolerant to vancomycin at concentrations 100's fold above the MIC. Thus PK/PD studies to investigate vancomycin levels in relation to clinical outcomes are urgently needed. However, an accurate and sensitive chromatographic method (HPLC) is lacking to analyze fecal vancomycin concentrations.

Goals: This work aimed to develop a simple and rapid HPLC assay to analyze fecal vancomycin concentrations in human clinical samples, and further to explore the distribution pattern of fecal vancomycin.

Methods: In the analysis platform of HPLC, the mixture of acetonitrile-water (10:90, 0.1% TFA) was used for the mobile phase with a flow rate at 0.3 ml/min and the detection wavelength of 205 nm. For clinical sample analysis, a total of 18 human fecal samples were collected, including one vancomycin-free control sample. Each unique fecal sample was divided into three aliquots between 20-150 mg. Vancomycin in each aliquot was extracted with the mixture acetonitrile-water (10:90, 0.1% TFA). The vancomycin extract together with a series of vancomycin standards (0.1-100 μg/ml) was applied to the developed HPLC assay.

Results: The developed method had a limitation of detection (LOD) at 0.1 μg/ml and a limitation of quantification (LOQ) of 0.4 μg/ml. The assay exhibited good linearity in the range of 0.4-100 μg/ml (R^2>0.99). Recovery from spiked fecal samples ranged from 85% to 110 %. Analysis of fecal vancomycin in the clinical samples demonstrated that fecal vancomycin concentrations ranged from undetectable to 1,944 μg/g. The variation coefficient of detection results among different aliquots ranged from 2%-16% (mean = 5%), showing high consistence in the fecal vancomycin level irrespective of various weight aliquots of the sample.

Conclusion: A sensitive and reliable HPLC assay was developed and suitable for measuring fecal vancomycin in human clinical sample. The analysis results of clinical sample further demonstrated the uniform distribution pattern of fecal vancomycin. Therefore, a single aliquot of fecal sample (between 20 and 150 mg) is sufficient for evaluating the vancomycin fecal concentration.

Acknowledgement: The grant support from University of Houston was greatly appreciated.

Poster #57

Epidemiology of Carbapenem-Resistant Enterobacteriaceae in a Large Academic Teaching Hospital in Houston, Texas

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Background: Carbapenem-resistant *Enterobacteriaceae* (CRE) have been classified as an urgent threat by the Centers for Disease Control and Prevention (CDC) since 2013. This study sought to understand the presence of CRE in various microbiology specimens and the mechanisms of resistance at our institution.

Methods: Consecutive clinical CRE isolates were prospectively collected from a single hospital in the Texas Medical Center between February 1, 2017 to March 23, 2020. CRE were identified daily by TheraDoc (Premier Inc., Charlotte, NC) and defined using the CDC definition found in the 2012 CRE Toolkit. Organism identification and minimum inhibitory concentration (MIC) determinations were performed by the Vitek 2 system (bioMérieux, Marcy l'Étoile, France), and MICs were interpreted using the 30th edition of the Clinical Laboratory Standards Institute M100 document or product package insert. The Streck ARM-D (Streck Inc., Omaha, NE) kits were used to detect and differentiate gene sequences from pure colonies. The Wilcoxon rank-sum test was used to compare MIC distributions between carbapenemase positive and negative isolates. All statistical analyses were performed using STATA, version 15.1 (StataCorp LLC, College Station, Texas).

Results: A total of 149 CRE isolates were collected during the study period. MIC and carbapenemase data were available for 113 (75.8%) and 83 (55.7%) isolates, respectively. The most common culture sites were urine (43.6%), lung (22.2%), and wound (14.1%). Twelve species were identified, the most prevalent of which were *Klebsiella pneumoniae* (42.3%), *Escherichia coli* (16.1%), and *Enterobacter cloacae* complex (15.4%). A carbapenemase gene sequence was detected in 32/83 (38.6%) isolates tested. MIC distributions were significantly higher in carbapenemase positive isolates for meropenem ($P < 0.0001$) and ertapenem ($P < 0.0001$). Conversely, MIC distributions did not differ for ceftazidime-avibactam, gentamicin, and sulfamethoxazole/trimethoprim ($P > 0.05$ for all).

Conclusion: In our prospective, single center, epidemiological study, the most common source of CRE was the urine 65/149 (43.6%). Of the 83 isolates tested, 32 (38.6%) contained a carbapenemase gene sequence, which is lower than previous epidemiological observations in the United States.

Poster #58

Multidrug-Resistant Klebsiella Pneumoniae as the Main Colonizing Organism in Patients from Intensive Care Units (ICU) in Two High Complexity Hospitals from Colombia

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Background: MDRO are a global public health threat associated with significant morbidity and mortality. Gastrointestinal and respiratory tract colonization by MDRO are well-recognized factors for development of invasive infections, particularly in critical-ill patients. There is a lack of data about rates of MDRO in high-risk patients in Colombia.

Goal: We aimed to investigate the epidemiology of MDRO-colonization in patients admitted to ICU in two high-complexity hospitals from Colombia.

Methods: 35 adult patients admitted to ICU from 2 high-complexity hospitals in 2 Colombian largest cities (October 2019 to March 2020) were included. Rectal and nasal swabs were collected on admission (day 0) and on day 7 of ICU stay or when an infection was reported. MDRO were recovered and identified using chromogenic media and PCR. Clinical data was collected using REDCap from eCRF. Demographic variables, Charlson (CCI), Pitt index (PBS), and final clinical outcomes were analyzed.

Results: 34 out of the 35 patients were colonized with Carbapenem-Resistant Enterobacteriaceae and *K. pneumoniae* (CR-Kp) was the predominant specie in 82% of patients. Further, co-colonization with CR-*E. coli* was found in 22 patients. Whereas, Methicillin resistant *S. aureus (*MRSA) was found in 5 patients. Most of patients were men (n=25) 71%; median age was 58 years. 26% of patients were admitted from home and 74% were considered hospital transfers. CCI and PBS scores were 1 (SD=2) and 3 (SD=3), respectively. Chronic obstructive pulmonary disease was the most common comorbidity found in 10 patients, of these 80% were colonized with both *P. aeruginosa* and CR-Kp. Antibiotic use was informed in 7 patients, and Beta-lactams were the most commonly agents. Cephalosporins, piperacillin-tazobactam, and carbapenems were prescribed in 86%, 43% and 28%, respectively. Invasive medical devices were present in all patients. Further, 8 out of 9 patients with mechanical ventilation had an association with CR-Kp. The mortality rate was 8.6%. Whereas, 63% of patients were discharged home, and 28% remained in ICU.

Conclusions: We found a high rate of MDRO carriers within ICU. Since, CRE and MRSA are endemic in hospitals in Colombia. Our findings recommend to perform MDRO active detection in our country.

Poster #59

Current Challenges and Future Directions of Antimicrobial Surfaces in the Era of COVID-19

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Background: The United States government had historically expressed great unenthusiasm regarding use of antimicrobial surfaces. The Center for Disease Control (CDC) stated in 2003, "no evidence is available to suggest that use of products treated with antimicrobial chemicals will make patients healthier or prevent disease." The Environmental Protection Agency (EPA), which regulates efficacy claims for antimicrobial surfaces, has not approved any claims of long-term efficacy against viruses. However, COVID-19 has reignited interest in use of antimicrobial surfaces, and numerous clinical trials have shown some materials exhibit efficacy against both viruses and bacteria, with one study showing a 58% reduction in infection rate when only 6 high-traffic surfaces were comprised of copper alloy (less than 10% of all surface area in the room).

Hypothesis/Goals: Our goal was to review recent literature regarding the efficacy of antimicrobial surfaces, as well as factors that promote or inhibit their utilization in real-world applications.

Methods: We performed a keyword search of medical literature using the search terms "antimicrobial surface" and "COVID-19". Relevant articles were reviewed and selected for inclusion into this report.

Results: We found substantial clinical evidence of efficacy for antimicrobial surfaces to dramatically reduce surface contamination and hospital infection rates. The CDC estimates that healthcare-associated infections affect 2 million patients annually and cost the healthcare system between 35.7 to 45 billion dollars per year. The pragmatic use of antimicrobial surfaces has the potential to dramatically reduce healthcare-associated infection rates and decrease healthcare costs.

Conclusions: Utilization of antimicrobial surfaces will likely increase, particularly in regard to copper alloys. Even prior to COVID-19, a 58% reduction in healthcare-associated infection rates would result in an estimated cost savings of up to 26.1 billion dollars. Copper alloys currently have EPA registration approvals for *S. aureus*, *Enterobacter aerogenes*, *E. coli* O157: H7, *Pseudomonas aeruginosa*, MRSA, and VRE. Studies have shown efficacy against the SARS-CoV-2 and EPA registration is a future probability. Technology is also available which allows the application of a copper coating onto existing surfaces, minimizing cost of deployment in both hospitals and high-traffic public areas. Future use of more exotic antimicrobial surfaces is less certain, with barriers including potential toxicity and environmental harm, as well as strict governmental standards pertaining to any claims of efficacy and clinical benefit. The COVID-19 pandemic will generate pressure on industry and government to work together in studying, approving, and deploying this technology in the interest of public health.

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Poster #60

Heteroresistance and Genome Sequencing of Pseudomonas aeruginosa in Cystic Fibrosis Patients

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Background: *Pseudomonas aeruginosa* (PA) is the leading cause of morbidity and mortality in cystic fibrosis (CF) patients. Heteroresistance (HR), in which members of a bacterial population display varying antimicrobial susceptibility testing (AST), can occur but is not well quantified or understood on phenotypic or genotypic levels.

Hypothesis/Goals: HR can be missed during standard AST in the laboratory. Prospective, additional AST could quantify and characterize otherwise unknown HR and whole genome sequencing (WGS) could identify potential genetic drivers of HR.

Methods: We prospectively collected 30 unique sputum samples from a group of 20 patients with CF (range of 1-5 samples per patient). In addition to standard AST on isolates selected by the laboratory (n = 1-3 isolates per patient), we collected additional isolates (n = 3-16 per sample), yielding a total of 267 additional isolates. We included only non-mucoid PA samples and performed AST on these isolates using 13 common antimicrobials and the 2019 CLSI breakpoints, yielding 3,471 additional drug-isolate results. We analyzed the data for the prevalence of HR and rates of HR across drugs, patients, and samples. We then calculated the number of instances where standard AST reported sensitivity to a given drug while resistant isolates were found on further AST. Lastly, we performed WGS on 145 isolates and used Fisher's exact test to measure association of gene variants to AST phenotypes.

Results: Median age was 39 (n=19). 47% of patients had the DelF508 CFTR mutation. Among the 267 isolates, 60% demonstrated HR with regard to at least one drug and 30% demonstrated HR to four or more drugs. Cefepime demonstrated the lowest rate of HR across patients (4.5% of isolates). Piperacillin-tazobactam, meropenem, and amikacin showed the highest rates of HR (15.7, 13.5, and 11.2% of isolates, respectively). AST results from additional isolates 'unmasked' resistance to at least one drug in 5 of the 30 samples. This was seen a total of 7 times in the 5 samples (meropenem n = 2 isolates, piperacillin-tazobactam n = 2, tobramycin n = 1, aztreonam n = 1, gentamicin n = 1). WGS revealed many unique variants that were associated with phenotypic resistance. This included mutations in the porin *oprD* that were significantly associated with meropenem resistance (p = 0.003).

Conclusion: Standard AST of sputum samples from CF patients can obscure a rich diversity of HR. HR to at least one agent was present in a majority of samples (60%) and HR to four or more agents was found in 30% of our samples. Sampling error during standard AST procedures can result in HR populations being reported as uniformly sensitive to a given agent, despite the presence of unsampled resistant isolates. This likely contributes to instances of clinical failure with reportedly effective drugs. Lastly, WGS of our samples revealed thousands of statistically significant genotypic associations with resistance to common antibiotics. These results have implications for both diagnosis and treatment of patients with PA infections and CF.

Acknowledgements: The Greenberg Lab and Infectious Diseases Biorepository

Poster #61

Subinhibitory Concentrations of Omadacycline Inhibit Staphylococcus aureus Hemolytic Activity in Vitro

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Background: In animal models of *Staphylococcus aureus* infection, α-hemolysin has been shown to be a key virulence factor. Treatment of *S. aureus* with subinhibitory levels of protein synthesis inhibitors can decrease α-hemolysin expression. Omadacycline, a novel aminomethylcycline antibiotic in the tetracycline class of bacterial protein biosynthesis inhibitors, is approved in the United States for treatment of community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI) in adults.

Objective: This study was performed to determine the durability of inhibition and effect of subinhibitory concentrations of omadacycline on *S. aureus* hemolytic activity.

Methods: All experiments used the methicillin-sensitive *S. aureus* strain Wood 46 (ATCC 10832), a laboratory strain known to secrete high levels of α-hemolysin. Minimum inhibitory concentrations (MICs) of omadacycline and comparator antibiotics (tetracycline, cephalothin, clindamycin, vancomycin, linezolid) were determined. Growth of *S. aureus* with all antibiotics was determined and the percentage of hemolysis assayed. "Washout" experiments were performed with omadacycline only.

Results: *S. aureus* cultures treated with 1/2 or 1/4 the MIC of omadacycline for 4 hours showed hemolysis units/10^8 CFU of 47% and 59% of vehicle-treated cultures, respectively. In washout experiments, treatment with as little as 1/4 the MIC of omadacycline for 1 hour decreased the hemolysis units/10^8 CFU by 60% for 4 hours following removal of the drug.

Conclusions: Omadacycline inhibited *S. aureus* hemolytic activity *in vitro* at subinhibitory concentrations and inhibition was maintained for ≥ 4 hours after removal of extracellular drug. The suppression of virulence factors throughout the approved omadacycline dosing interval, in addition to the *in vitro* potency of omadacycline, may contribute to the efficacy of omadacycline for ABSSSI and CABP due to virulent *S. aureus*. This finding may apply to other organisms and other virulence factors that require new protein synthesis to establish disease.

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Poster #62

Parallel, Endemic Dissemination of Carbapenem Resistant CG307 and CG258 Klebsiella Pneumoniae Lineages with Unique Accessory Genomes in Houston, TX

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Poster #62

Background: Carbapenem-resistant *Klebsiella pneumoniae* (CR*Kp*) remain public health threats in hospital settings. Carbapenem resistance primarily occurs in CR*Kp* through the acquisition of carbapenem hydrolyzing enzymes known as carbapenemases (*e.g., Klebsiella pneumoniae* carbapenemase [KPC]). A substantial number of invasive and serious infections due to the high-risk *K. pneumoniae* clonal groups CG258 and CG307 have been identified in the Houston, TX region. The extent of which adaptive gene features, *e.g.* antimicrobial resistance (AMR) genes and virulence genes, are shared across CG258 and CG307, remain largely unknown.

Hypothesis/Goals: Determine the extent of genomic sharing between the two highly endemic lineages CG258 and CG307 co-circulating in Houston, TX

Methods: 95 CR*Kp* isolates were collected from a large, Houston hospital network. Additionally, 12 CG307 isolates matched geographically with 12 CG258 isolates from the larger CRACKLE-2 study were included in the analyses. Oxford Nanopore Technologies long-read and Illumina short-read sequencing were utilized to resolve complete CR*Kp* genomes. This data was used to perform a comprehensive comparative genome analysis using custom bioinformatic pipelines to dissect the accessory genome of co-circulating CR*Kp* isolates. Accessory genome clustering analysis such as t-distributed stochastic neighbor embedding (t-SNE) was performed. An inverse probability weighted (IPW) Desirability of Outcome Ranking (DOOR) analysis was used to assess potential clinical outcome differences between patients colonized or infected with CG258 compared to CG307.

Results: Through dimension reduction analysis of the accessory genome, two segregated clusters of CG258 (n = 49) and CG307 (n = 50) with clearly defined boundaries were identified. Agglomerative hierarchical clustering of plasmid content indicates clustering by clonal group in particular for CG258 with a less defined separation between CG307 and other co-circulating clonal groups. The accessory genome content of CG307 is more similar to other CGs, in particular with CG147, as compared to the CG258 lineage. Transfer efficiency to recipient *Escherichia coli* of the unique plasmid type associated with the Houston CG307 isolates (pCG307_HTX) that harbored the $bla_{\text{KPC-2}}$ carbapenemase was comparable to the CG258 associated pKpQIL plasmid associated with $bla_{\text{KPC-2}}$ carriage. The IPW-DOOR analysis indicates that a randomly selected patient colonized/infected with CG307 had a 64% (95% CI: 53-73%) higher probability of a more favorable outcome compared to a patient colonized/infected with CG258 30-days after hospital admission.

Conclusion: CG258 and CG307 possess distinct, independent accessory genomes suggesting unique clonal expansion events that led to endemic persistence in the Houston, Texas region. While both lineages predominantly develop carbapenem resistance through acquisition of bla_{KPC} harbored on Tn*4401a* transposons, each lineage has a unique composition of plasmid vectors responsible for horizontal gene transfer of these carbapenemase vectors. Furthermore, there is greater evidence of accessory genome sharing between CG307 and other CGs as compared to CG258 that has a much more closed accessory genome. IPW-DOOR analysis suggests patients with CG307 colonization/infection may have better outcomes 30-days after hospital admission compared to patients with CG258 colonization/infection.

Poster #63

Epidemiology of Penicillin Allergy Labels in the Pediatric Primary Care Setting

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Background: Penicillin allergy labels heavily influence a clinician's selection of antibiotics, yet most children labeled as penicillin-allergic do not have a type 1 hypersensitivity allergy. Initiatives to address erroneous penicillin allergy labels have been conducted in hospitals, but many children are never hospitalized, and most antibiotics are prescribed outpatient. The epidemiology of penicillin allergy labeling in the pediatric primary care setting is not fully understood.

Goals/Hypothesis: The purpose of this study is to describe the epidemiology and factors associated with penicillin allergy labeling across two of the largest pediatric primary care networks in the United States. Based on a review of adult literature, we hypothesized that 5-10 % of children will be labeled as penicillin allergic but that there will be variation in rates of labeling among different clinics. We also hypothesized that most patients will be labeled by age 3 years.

Methods: We described penicillin allergy labelling of children receiving care at one of 57 Texas Children's Pediatrics (TCP) primary care pediatric clinics or 31 Children's Hospital of Philadelphia (CHOP) care clinics. Children were included in the two-center birth cohort if they were born between Jan 1, 2010 (TCP) or Jan 1, 2004 (CHOP) and June 30, 2020, had a primary care clinical encounter within the first two weeks of life and at least two additional encounters in the first year of life. Patient demographics, encounters, medications, and allergy data were extracted from the electronic medical record. Patients were categorized as penicillin-allergic or not based on their allergy tab label. Demographics were reported as frequencies and continuous variables as means with 95 % confidence intervals.

Results: 385,845 children met inclusion criteria for the birth cohort. The mean prevalence of penicillin allergy labels was 5.8 % (5.1% TCP; 6.6 % CHOP). Rates of penicillin allergy labeling by clinic ranged from <1 to 7.7 %. There were differences in patient demographics between penicillin-allergic and non-allergic groups. The mean age of penicillin allergy labels at the two centers was 2.16 years (95 % CI 2.13, 2.19)**.** Over 90 % of children were labeled by 4 years of age.

Conclusions: Overall, 5.8 % of children across more than 80 pediatric primary care practices were labeled as penicillin allergic. Rates of allergy labeling varied across practice, and most children were labeled by 4 years of age. Future work should determine the predictors of inappropriate allergy labeling, its impact on first-line antibiotic prescribing, and clinical outcomes.

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Poster #64

Antibacterial Activity of Extracellular Metabolites Secreted by Model Moss Physcomitrella Patens

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Background: The secondary metabolites and secreted components of Bryophytes are of particular interest both in fundamental research and applied sciences. Many of these compounds are unique and considered as the promising natural antibacterial agents.

Hypothesis/Goals: The aim of this research is to analyze the antibacterial activity of secreted metabolites produced by model moss *Physcomitrella patens*.

Methods: *P. patens* (Gransden ecotype) exudates were prepared for the analysis of the antibacterial activity of the extracellular metabolites. Moss protonema was grown in liquid BCD medium for 1, 2, and 4 weeks in the standard conditions. After incubation period the cultural medium was collected, freeze-dried in lyophylizer and dissolved in sterile N-free deionized H_2O in the final concentration 100 mg/ml. The disk diffusion method (DDM) and MIC (minimum inhibitory concentration test) were used to measure antibacterial activity of exudates. Extracellular metabolites were analyzed for their stability for the heating and proteinase K treatment. For heating analysis the exudates' samples were boiled for 10 min, for the proteolysis stability samples were treated with 100 ug/ml of proteinase K and incubated for 3 h at 37 °C. Treated samples were used in MIC analysis. Gram-positive and gram-negative bacteria were used as test-cultures. All samples were tested in triplicates.

Results: The antibacterial activity against gram-positive bacteria was detected in *P. patens* (Gransden) exudates. The highest antibacterial activity was shown against gram-positive bacteria *Staphylococcus aureus* ATCC25923 in the 2 and 4-week old fractions of secreted metabolites (the average inhibition zones were 13,17±1,27 mm and 12,97±1,27 mm, respectively). MICs against *S. aureus* ATCC25923 were 25 mg/ml for 2-week old exudates and 12,5 mg/ml for 4-week old exudates. MICs of 4-week old exudates were 12,5 mg/ml against *Streptococcus pyogenes* and 50 mg/ml against *Enterococcus faecalis*. Exudates in concentration 12,5 mg/ml lost the growth inhibitory activity against *S. aureus* after treatment with Proteinase K but maintained activity in concentration 25 mg/ml. Boiling for 10 min led to the lost of the antibacterial activity in any concentrations of exudates. No activity against Gram-negative bacteria was detected for *P. patens* exudates.

Conclusions: Thus, our data confirm the presence of a significant antimicrobial potential of *Physcomitrella patens* extracellular metabolites against Gram-positive bacteria. Further experiments will focus on the identification of the individual active components of exudates and characterization of metabolites also basing on their stability properties.

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Poster #65

Multi-omic Analysis of Host-microbiome Signaling Associated with Probiotic Efficacy in Traumatic Brain Injury

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Background: Traumatic Brain Injury (TBI) is a major infection risk and causes death and disability, representing one of the most prevalent injury types sustained by the worldwide population. For patients, not only is the initial blunt trauma damaging, but also the subsequent exacerbating cellular and molecular damage. This includes neuronal death and tissue loss, neuroinflammation, gliosis and cell infiltration, and blood brain barrier (BBB) breakdown and edema which constitutes a major risk for infection. In terms of treatment, recent studies have shown potential in the gut-brain axis, as the gut microbiome modulates neuroinflammation and BBB permeability. Notably, there appears to be reduction in *Lactobacillus* spp. following TBI.

Hypothesis/Goals: It was predicted that giving probiotics of mostly *Lactobacillus* composition to TBI-modeling mice could help to ameliorate TBI-induced neuropathology, including neuroinflammation that is exacerbated by the associated gut dysbiosis.

Methods: Mice were given either Sham Surgery or Controlled Cortical Impact (CCI Surgery), then treated with either a pan-bacteria cocktail (of *Lactobacillus* composition) or the vehicle. Fecal samples collected at baseline, 3 days post injury (DPI), and 35 DPI were used for 16S rRNA analysis and shotgun metaproteome analysis. Microbiome comparison was performed utilizing STAMP software, and host response was detected by Qiagen Ingenuity Pathway Analysis.

Results: Comparison of microbiome composition at 35 DPI using 16S data showed no significant difference in abundance of either the Lactobacillaceae family or the Lachnospiricae family. Instead, the only significant alterations were a decrease in abundance of Rikenellaceae and an unclassified Bacteroidales in the CCI Vehicle group compared to the CCI Pan-Bacteria group. Metaproteomic data measuring metabolically active microbiota communities demonstrated markedly different results when compared to the 16S findings. Notably, significant abundance of all *Lactobacillus* species from the pan-bacteria cocktail in the CCI modeling treated group were evident at both 3 and 35 DPI. Additionally, host proteome signals evaluated at 3 DPI using Ingenuity Pathway Analysis revealed signs of clinical improvement in the CCI mice that were treated with the *Lactobacillus* pan-bacteria cocktail. The pathways that were most differentially expressed between the treated and untreated CCI groups included mitochondrial dysfunction, oxidative phosphorylation, sirtuin signaling pathway, remodeling of epithelial adherens junctions, and serotonin degradation.

Conclusions: Investigating the metabolically active microbiome in disease states associated with gut dysbiosis provides a very different perspective of bacterial community shifts, including detection of potentially therapeutic probiotics. Unique insight into probiotic-induced host signals associated with clinical efficacy is also evident using a metaproteomics approach.

Poster #66

In situ Structure of the AcrAB-TolC Efflux Pump at Subnanometer Resolution

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Background: Antibiotic resistance is an emerging crisis in worldwide healthcare. In Gram-negative bacteria, a prominent resistance mechanism is provided by multidrug efflux pumps, which actively expel a broad range of substances, including antibiotics. Tripartite efflux pumps span the inner and outer membranes, passing through the periplasm. This set of cellular environments is impossible to replicate *in vitro*, making structure/function studies extremely challenging.

Goals: To achieve a high resolution *in situ* structure of the AcrAB-TolC efflux pump and to better understand the native state and structure of these pumps.

Results: We use high resolution cellular electron cryotomography to visualize the structure of the assembled AcrAB-TolC within intact *Escherichia coli* cells at a 7 Å resolution. The resulting sub-nanometer cryo-ET structures show the detailed architecture of the assembled complex embedded into two lipid bilayer milieus, including unexpected limited penetration of the outer membrane. Interactions with the inner membrane enable cross-talking between AcrB and channel TolC through AcrA.

Conclusions: These findings *in situ* suggest that assembly in the native cellular environment is critical in the pump activation mechanism, where the allosteric activating signal is triggered by the observed alternate binding of AcrA to the lipid membrane and AcrB porter domain. We establish a platform for efflux pump structural studies in bacteria that demonstrates that high resolution *in situ* studies can yield critical information in understanding complex assemblies function and provide a path for future studies of these membrane complexes under true native conditions.

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Poster #67

Evaluating Phage Therapy for the Treatment of Urinary Tract Infection

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Background: The estimated 7 million urinary tract infections (UTIs) that occur annually in the United States represent the most common cause of outpatient antibiotic prescriptions. The majority of UTIs are caused by uropathogenic *E. coli* (UPEC) and resolve with standard antibiotic treatment. However, a subset of those treated will have recurrence, requiring additional antibiotic treatment. Because antibiotics negatively impact the healthy microbiota and can contribute to the development of antibiotic resistance, alternative treatments are urgently needed. Although bacteriophages (phages), viruses that selectively infect bacteria, are an appealing targeted therapy for many bacterial pathogens, the efficacy of phage therapy in treating UTIs has not been well-defined. Classically, phage-bacteria interactions have been studied in bacteriologic media, or host environments such as the blood. These environments do not represent the unique pH, salinity, and nutrient conditions present in urine.

Hypothesis/Goals: In this study, we aimed to characterize the impact that these unique conditions have on the killing ability of bacteriophages.

Methods: To do this, we screened a variety of phages against UPEC isolates, altering culture media and concentration of phage. Phage-mediated killing was measured via optical density over an 18-hour timeframe.

Results: Our results demonstrate that urine is broadly inhibitory towards phage-mediated UPEC killing and that phage resistance develops in several UPEC strains within the first six hours of infection, leading to an "OD rebound" observed by 18 hours. However, *in vivo*, these mutations appear to negatively impact the bacteria's ability to colonize and cause inflammation in the bladder of infected mice.

Conclusions: Ongoing research seeks to classify the genetic bases underlying resistance to individual phages and to further define the inhibitory nature of urine. These findings will identify phages capable of being used *in vivo* and will contribute to the development of phage-phage and phage-antibiotic cocktails for treating and preventing UTI.

Poster #68

Urine Culture High Contamination Rates call into Question the Gold Standard for Urinary Tract Infections

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Objectives: Urine cultures are the most common microbiological tests in the outpatient setting and heavily influence treatment of suspected urinary tract infections (UTI). Antibiotics for UTI are usually prescribed on an empiric basis in primary care before the urine culture results are available. However, culture results may be needed to confirm a UTI diagnosis and to verify that the correct antibiotic was prescribed. While urine cultures are considered as the gold standard for diagnosis of UTI, cultures can easily become contaminated during collection. We determined the prevalence and predictors of contaminated urine cultures in two adult safety net primary care clinics. We also studied antibiotic use associated with contaminated and no growth cultures.

Methods: This study was a retrospective chart review of visits with provider suspected UTI where a urine culture was ordered (November 2018-March 2020). Patient demographics, culture results and prescription orders were captured for each visit. Culture results were defined as no culture growth, contaminated (i.e., mixed flora, non-uropathogens, or >2 bacteria isolated on culture), low counts (growth between 100 and 100,000 cfu/ml), and high counts (≥100,000 cfu/ml). Univariate and multivariable logistic regression models were used to identify factors associated with contaminated culture results.

Results: There were a total of 1265 visits with urine cultures, of which 264 (20.9%) had no growth, 694 (54.9%) were contaminated, 159 (12.6%) were low counts, and 148 (11.7%) were high counts. Female gender and pregnancy were independently associated with contaminated cultures (adjusted Odds Ratio (aOR), 95% confidence interval (CI) 4.42 (3.07-6.47) and 6.67 (4.26-10.58) respectively, P<0.001). However, patients with diabetes were less likely to have contaminated cultures (aOR 0.71 (0.52-0.97), P=0.03). Of 264 cultures with no growth, 36 (14%) were prescribed an antibiotic. Of 694 contaminated cultures, 153 (22%) were prescribed an antibiotic.

Conclusions: More than half of urine cultures were contaminated in primary care clinics, thus calling into question current methods of patient instruction and preparation for urine culture collection. Female gender and pregnancy were associated with contaminated culture results. Reduction of contamination should improve patient care by providing a more accurate record of the organism in the urine (if any) and its susceptibilities, which is relevant to managing future episodes of UTI in that patient. Optimizing urine collection represents a diagnostic stewardship opportunity in primary care.

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Poster #69

Back to The Future: Increasing Penicillin Susceptibility among Methicillin-Susceptible Staphylococcus aureus Osteoarticular Infections in Children

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Introduction: *Staphylococcus aureus* is a common bacterial pathogen with the potential for causing serious disease. Starting in the late 1940s-1950s *S. aureus* isolates acquired resistance to penicillin largely through the acquisition of β-lactamases. In recent years, some centers have described an increase in the proportion of methicillin susceptible *S. aureus* (MSSA) which are also susceptible to penicillin (PSSA). There are little data on the prevalence or clinical significance of PSSA in children. Acute hematogenous osteoarticular infections (AHOAIs, including osteomyelitis and septic arthritis) are the most common manifestation of invasive *S. aureus* disease in children. We investigated the prevalence of penicillin susceptibility among MSSA AHOAI isolates at two children's hospitals.

Methods: MSSA AHOAI isolates were obtained through surveillance studies at Texas Children's and St. Louis Children's Hospitals from 1/2011- 12/2018. All isolates underwent PCR for *blaZ* β-lactamase, PVL genes and *agr* group. All *blaZ* negative isolates then underwent penicillin susceptibility testing using macrobroth dilution. Isolates which were *blaZ* negative and had a penicillin MIC ≤ 0.125 μg/ml were regarded as PSSA. These studies were performed in the research context and results were not available to treating providers. All PSSA also had MICs determined for cefazolin and cephalexin. Temporal trends in PSSA were examined.

Results: 285 unique isolates were available and included in the study. The median patient age was 9.3 years (IQR: 5.7-12.2). Overall, 13 isolates were found to be penicillin susceptible (4.5%). No PSSA isolates were detected in the first half of the study period but increased yearly thereafter; by the final study year 14.6% of isolates were PSSA (p=0.02, **Figure 1**). PSSA were similar to penicillin-resistant isolates in terms *agr* group and PVL carriage as well as long term orthopedic outcomes. Patients with PSSA isolates were slightly older than those with resistant isolates (median age 12.2 years vs. 9.1 years, p=0.08). No patient was treated with penicillin. For PSSA, the penicillin MIC_{90} (0.06 μg/ml) was much lower than that for cefazolin or cephalexin (**Figure 2**).

Conclusions: The proportion of MSSA that are PSSA appears to be increasing among AHOAI isolates in children. Overall, PSSA isolates are associated with a similar phenotype as penicillin-resistant isolates. Penicillin susceptibility testing may serve as an avenue for future stewardship intervention in staphylococcal infections. Further research is needed to understand the comparative efficacy of penicillin vs. antistaphylococcal penicillins or cephalosporins in the treatment of invasive MSSA.

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Poster #70

Development of Bacteriophages with Anti-Biofilm Properties as Novel Treatment for Catheter-Associated Urinary Tract Infections

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Background: Urinary tract infections (UTIs) are one of the most common bacterial infections and an important public health issue. Compared to uncomplicated UTIs, catheter-associated urinary tract infections (CAUTIs) have higher morbidity and mortality and are the main cause of gram-negative bacteremia. The most common causative agent of UTIs is *Escherichia coli.* The formation of biofilms in the urinary tract and on urinary catheters, and the increasing rate of antibiotic resistance found in community and nosocomial *E. coli* strains makes treatment of *E. coli* UTIs extremely challenging.

Hypothesis/Goals: Bacteriophages (phages or Φ), are ubiquitous viruses that exclusively infect and kill bacteria irrespective of their antibiotic sensitivity. Phages are active in human urine and can produce enzymes that degrade bacterial extracellular polysaccharides (EPS), such as those in biofilms. We hypothesize that phages have unique genetic and molecular elements that make them suitable as a treatment option for biofilm-associated infections, including CAUTI. The objective of this work is to discover and characterize phages that kill *E. coli* growing in biofilms using an *in vitro* model of CAUTI.

Methods: *E. coli* strains were obtained from urine specimens of persons with spinal cord injury. Phages (28) were obtained from previously characterized phage libraries. *E. coli* biofilms were grown for 24 - 48 hours at 37°C in Tryptic Soy Broth (TSB) or pooled human urine, washed and treated with 10^7 PFU/mL of phage for 24 hours. At this point, the biofilms were washed again and stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to determine the metabolic output of live cells within the biofilms by measuring absorbance (540 nm) of the resulting formazan crystals.

Results: *E. coli* phages were initially screened on pre-formed biofilms of *E. coli* DS515 grown in rich media (TSB). Of the 28 phages tested, 8 (28.6%) decreased biofilm viability by >50%, 9 (32.1%) reduced biofilm viability by 25-50%, and 11 phages (39.3%) did not cause a significant reduction in biofilm viability. Subsequently, a similar phage screen was performed against two *E. coli* clinical strains (DS515 and DS552) grown as biofilms in human urine. Phages HP3, ES17, ES21, ES26, 6915 and 6950 each decreased biofilm viability by >85% when added individually to 48-hour biofilms in human urine of both *E. coli* strains. Four of these 6 phage with anti-biofilm activity form plaques with halos when plated on bacterial lawns. The presence of halos suggests that enzymatic activity against the bacterial EPS might be associated with biofilm destruction by phage.

Conclusions: We have identified six phages (HP3, ES17, ES21, ES26, 6915 and 6950) with anti-biofilm activity against *E. coli* in human urine. These phages may have therapeutic potential against CAUTIs, and our future studies will test their efficacy *in vivo.*

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Poster #71

Adoption of the Revised CLSI Fluoroquinolones Breakpoints for Gram-negative Bacteria

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Background
Despite the multiple safety warnings related to fluoroquinolones (FQs) treatment, their use remains unavoidable in several occasions due to their broad spectrum of coverage including activity against multi-drug resistant glucose non-fermenting Gram-negative bacteria such as *Pseudomonas* spp., and high oral bioavailability.

Hypothesis/Goals
The Clinical and Laboratory Standards Institute (CLSI) has lowered the FQs minimal inhibitory concentrations (MICs) breakpoints for *Salmonella* spp. in 2012 and 2013, and for the *Enterobacterales* and *P. aeruginosa* in 2019. We aim to explore the number of hospitals that adopted the revised breakpoints.

Materials
We conducted a cross-sectional phone-based survey querying the 43 microbiology laboratories that serve 100% of the acute care and long-term hospitals in Connecticut to determine use of revised FQs MIC breakpoints for Gram-negative bacteria.

Results
Six laboratories refer antimicrobial susceptibility testing to another local hospital microbiology laboratory or to a national reference laboratory. Thus, we obtained information about the study question from a total of 37 microbiology laboratories. Eight laboratories (21.6%) were affiliated to university hospitals and 29 (78.4%) were community-based. Microscan Beckman coulter MicroScan was the most common antimicrobial susceptibility test method used in 15 (40.6%) microbiology laboratories followed by BioMérieux Vitek 2 in 13 (35.1%) laboratories. Four laboratories (10.8%) only adopted the revised CLSI FQs breakpoints for *Enterobacterales, P. aeruginosa*, and *Salmonella* spp, 5 (13.5%) implemented the revised breakpoints for *Enterobacterales* and *P. aeruginosa* but not for *Salmonella* spp., and 8 (21.6%) laboratories adopted the revised CLSI breakpoints for *Salmonella* spp. but not for *Enterobacterales* and *P. aeruginosa*.

Conclusions
The use of outdated CLSI breakpoints for FQs against Gram-negative bacteria remains common in the microbiology laboratories. There is an urgent need to mitigate the impact of using the outdated FQs breakpoints and reporting false susceptibility to FQs.

Acknowledgements
None

Poster #72

Non-carbapenemase Producing Organisms with CTX-M Gene Amplification Account for Majority of Invasive Carbapenem-resistant Enterobacterales bacteremia in Immunocompromised Patient Population

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Background: Carbapenem-resistant Enterobacterales (CRE) are considered urgent antimicrobial resistance threats. CRE mechanism research largely focuses on the characterization of carbapenem hydrolyzing enzymes known as carbapenemases. However, non-carbapenemase-producing carbapenem-resistant Enterobacterales (non-CP-CRE) are increasingly detected in hospital settings with mechanisms of carbapenem resistance in such organisms poorly understood.

Hypothesis/Goals: Determine the proportion of non-CP-CRE among diverse CRE species in a highly immunocompromised population and characterize their carbapenem resistance mechanisms.

Methods: We analyzed all CRE bloodstream isolates prospectively collected from July 2016 to March 2020 at MD Anderson Cancer Center in Houston, Texas. Carbapenem resistance was defined as any isolate having at least intermediate resistance to ertapenem, meropenem, or imipenem based on CLSI breakpoint guidelines. Minimum inhibitory concentrations (MICs) were determined by Etest through the MDACC clinical laboratory. Study isolates were subjected to whole genome sequencing using short-read Illumina sequencing with the NextSeq 500 platform. A suite of bioinformatic tools was used to determine species identification, multi-locus sequence typing, antimicrobial resistance (AMR) genes, and potential AMR gene copy number amplification.

Results: There were 79 CRE isolates that met our study criterion. The most common organisms detected were *Escherichia coli* (37/79; 46.8%), *Klebsiella pneumoniae* (27/79; 34.2%), and Enterobacter spp. (7/79; 8.9%). The most common identified carbapenemases in our population were KPC-2 (n = 8), OXA variants (n = 5), and NDM variants (n = 5). Nevertheless, the majority of our isolates (62/79; 78.5%) were non-CP-CRE isolates. *E. coli* isolates were 83.8% (31/37) non-CP-CRE with the most common carbapenem resistance (CR) mechanism (29/31; 87.1%) involving the presence of an ESBL and/or outer membrane porin disruption. The most common ESBL identified were CTX-M variants (25/29; 86.2%) with $bla_{\text{CTX-M}}$ copy number amplification (≥~2 copies) present in 68% of these *E. coli* isolates. The non-CP-CR *K. pneumoniae* isolates (20/27; 74.1%) were almost exclusively $bla_{\text{CTX-M-15}}$ carriers (18/20; 90%) of which

72.2% (13/18) had multiple *bla*$_{\text{CTX-M-15}}$ copy numbers. Of these isolates harboring *bla*$_{\text{CTX-M-15}}$, 77.8% (14/18) had a concomitant OmpK35 and/or OmpK36 disruption.

Conclusions: The vast majority of CRE bloodstream isolates in this immunocompromised population have non-CP-CRE mechanisms. Our short-read sequencing approaching showed that gene amplification of a CTX-M variant with concomitant porin disruption was strongly associated with non-CP-CRE. We are currently applying long-read sequencing technology to these isolates to determine mechanisms of gene amplification and porin disruption leading to the non-CP-CRE phenotype.

Poster #73

Characterization of the Antimicrobial Susceptibility Patterns and Virulence Mechanisms Promoting Staphylococcal Medical Device Infections

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Background: Nearly 10 million women have breast implants worldwide and an additional 300,000 are placed annually in the US for cosmetic and reconstructive purposes. Problematically, breast implant-associated infections (IAIs) are the most common complication following device placement (2%-29% of cases) and can result in significant morbidity, including tissue necrosis and disfigurement. These infections are difficult to treat as they often result in chronic, biofilm-associated diseases that exhibit increased recalcitrance to antimicrobials and/or the host immune system. Thus, IAIs are a highly relevant aspect of women's health. To combat these infections major efforts, including the administration of prophylactic antibiotic pocket irrigants, have been implemented, often with conflicting results. Importantly, the most common etiologic agent causing IAI is *Staphylococcus epidermidis* (SE), which historically was not considered a pathogen. Thus, studies investigating the mechanisms that promote SE IAI remain lacking. Understanding the host-pathogen-device interactions that promote IAI may lead to the development of better prevention and treatment strategies.

Goals: The goals of this study are to i) determine antibiotic susceptibility patterns of SE strains isolated from breast implants and ii) identify virulence mechanisms that promote IAI.

Methods: To define the mechanisms that promote SE IAI, 4 SE strains were isolated from women requiring explanation of breast implants due to complications who were consented and enrolled in our study. Minimum inhibitory concentration assays were performed using antibiotics commonly used in pocket irrigants to determine the antimicrobial susceptibility patterns. Additionally, we assessed these strains for i) the carriage of specific virulence factors, including adhesins, ii) the ability to adhere to different surfaces, including breast implants; ii) the requirements for biofilm formation. Lastly, a mouse model of IAI was developed to investigate the host-pathogen interactions that promote infection.

Results: These studies indicate that all SE strains were sensitive to gentamicin and cefazolin, but resistant to bacitracin. Additionally, all breast implant-associated SE (BIS) isolates encoded *sdrF* and a majority encoded *sdrG*, which are adhesins that interact with the host proteins collagen and fibrinogen, respectively. Furthermore, BIS adherence and biofilm formation were enhanced in the presence of collagen, but not fibrinogen. Lastly, the mouse model demonstrates that SE requires the breast implant to alter the environment to cause disease.

Conclusions: Together with our previous studies indicating that collagen is deposited on clinically collected patient implants, the conserved carriage of *sdrF* suggests that SE-collagen interactions may promote IAI. IAIs are a dreaded complication following device placement, as treatment options are limited. This study highlights SE-host interactions that may be targeted for the development of non-antibiotic therapies.

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Poster #74

Management of Staphylococcus aureus Bacteremia Among Adult Infectious Disease Specialists in Latin America: A Wide Variation in Treatment Approaches

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Background: Several studies have suggested that key interventions such as early active antimicrobial therapy and echocardiography are critical for the appropriate management of SAB. Among them, involvement of ID specialists has shown a major positive impact in the outcomes of SAB. However, recent data from the US suggest that there is high variability in the management of SAB among different ID specialists. Data regarding management of SAB in developing regions such as Latin America are scant.

Goals: We aimed to evaluate the consistency in the management of SAB among ID specialists across five Latin American countries

Methods: Management of SAB was assessed using an anonymous online survey electronically sent to ID physicians from Argentina, Brazil, Colombia, Chile, and Peru between June to November, 2019. The survey consisted of 14 multiple-choice questions related to diagnosis and management of SAB.

Results: A total of 654 surveys were received. The most critical findings were: that 69% of the ID doctors reported ordering and performing follow-up blood-culture and 75% carried echocardiography as routine in patients with SAB. Clinicians who preferred not to perform follow-up blood cultures were also more likely to prefer not to perform an echocardiographic assessment (OR 4.08, p=0.02). For the management of methicillin-susceptible *S. aureus* (MSSA) bacteremia, respondents overwhelmingly preferred oxacillin/cloxacillin (68%) as opposed to cefazolin/cephalothin (22%) (excluding Argentina where oxacillin/cloxacillin are not available). Conversely, for the management of methicillin-resistant *S. aureus*

bacteremia, ID LA physicians equally prefer either vancomycin (39%) or daptomycin (39.3%). Among subjects preferring daptomycin, 75% of report to use doses of 8-10 mg/kg/d. When dealing with a persistent SAB (i.e. > 7d of therapy) while on vancomycin, 70% of ID physicians in LA preferred to switch to daptomycin alone or in combination with β-lactams. ID specialists with < 10 years of practice were more likely to perform follow up blood cultures ($p<0.001$) and echocardiographic assessment ($p=0.05$), as compared to those with > 10 years of practice. Similarly, the former also i) preferred 14 days of IV therapy over shorter durations ($p<0.001$) to manage uncomplicated SAB, ii) were more likely to choose daptomycin for the management of MRSA bacteremia ($p=0.043$), and iii) were less likely to consider MRSA in a blood culture as a contaminant ($p=0.002$).

Conclusions: There is high variability in the management of SAB among ID clinicians from five Latin American countries. ID clinicians with <10 years of clinical practice were more likely to prefer management strategies that agree with current guidelines or with the most updated literature.

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Poster #75

Use of a DNA-Encoded Chemical Library Approach to Identify Inhibitors for the OXA-48 Carbapenemase

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Resistance to the highly prescribed β-lactam antibiotics is largely mediated by β-lactamases, bacterial enzymes that hydrolyze the pharmacophoric β-lactam ring of these antibiotics. A variety of unique β-lactam antibiotics have been developed to treat bacterial infections, but their extended use leads to the emergence of β-lactamase variants capable of hydrolyzing them. β-lactamase inhibitors have been created to restore effectiveness to β-lactam antibiotics, but most available inhibitors contain a β-lactam ring, making them vulnerable to hydrolysis and rapid resistance. Oxacillinase-48 (OXA-48) is a problematic β-lactamase that hydrolyzes nearly all β-lactam antibiotics including carbapenems, the last resort class of β-lactam antibiotics. Avibactam is the only clinically available non-β-lactam inhibitor and the only effective inhibitor against OXA-48. Due to the prevalence of OXA-48 in the clinics, there is a need for novel inhibitors to combat this public health threat. *We aim to develop a novel non-β-lactam OXA-48 inhibitor, unique from Avibactam, that will ultimately increase the β-lactam antibiotic susceptibility of bacteria expressing OXA-48*.

A DNA-encoded library (DEL) approach was used to rapidly and cost-effectively identify compounds that bind OXA-48. DELs consist of compounds that are tagged with unique DNA barcodes. These compounds can be screened by the millions against a target protein, all at once as binders can later be identified by sequencing the barcodes. With this approach, we screened over a billion non-β-lactam compounds against OXA-48 and discovered our lead compound CDD-97, which exhibits sub-micromolar potency against OXA-48 (K_i = 0.58 ± 0.1 μM). The x-ray crystal structure of CDD-97 in complex with OXA-48 was determined to aid in the design of new versions with increased potency. The most potent inhibitors found were also tested against bacteria expressing OXA-48 to determine whether the inhibitors could make the bacteria more susceptible to ampicillin, a β-lactam antibiotic rapidly hydrolyzed by OXA-48. However, the compounds showed no significant activity, likely due to low membrane permeability or efflux pumps. The combined structural and *in vivo* data is being used to design fragments of CDD-97 that may have increased permeability and can later be modified for increased potency. This process has rapidly provided insights on OXA-48 inhibition and completion of this project will further contribute to knowledge on OXA-48 and general β-lactamase inhibition, which may aid the discovery of a clinically useful OXA-48 inhibitor.

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Poster #76

Discordance of Ceftriaxone and Cefepime MICs in Streptococcus mitis/oralis Isolates at The University of Texas MD Anderson Cancer Center

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Background: *Streptococcus mitis/oralis* are the 2nd leading cause of bloodstream infections in cancer patients and thus a major driver of broad-spectrum antimicrobial use. β-lactams are the drugs of choice for *S. mitis/oralis* infection. Although β-lactam resistance does occur in *S. mitis/oralis*, there is a dearth of knowledge regarding resistance breakpoints for these organisms. For example, current CLSI guidelines set the same minimum inhibitory concentration (MIC) breakpoint for resistance of 1 µg/mL for ceftriaxone (CRO) and cefepime (FEP) for *Streptococcus mitis/oralis*. This FEP breakpoint is derived arbitrarily from that of CRO, and the prevalence of discordant susceptibility is unknown.

Hypothesis/Goals: We sought to determine the discordance of CRO and FEP MICs in *S. mitis/oralis* bloodstream isolates. We hypothesized that the distribution of FEP MICs is higher compared to CRO for *S. mitis/oralis* which could indicate an incorrect resistant breakpoint.

Methods: There were 243 *S. mitis/oralis* isolates identified from blood cultures from August 2018 to August 2020. Identification of the isolates was done using MALDI-TOF technology (VITEK-MS; bioMerieux Inc, Durham, NC) and the susceptibility testing against CRO and FEP was by ETEST (bioMerieux Inc, Durham, NC). Essential agreement (EA) was defined as agreement within one dilution between CRO and FEP MICs. Categorical agreement (CA) was defined as agreement in interpretive results [classifying an isolate susceptible or resistant] between CRO and FEP. Clinical isolates from the same patient were excluded if it was recovered within 1 week. Thus, 4 isolates were excluded, resulting in 239 unique *S. mitis/oralis* isolates included in the analysis.

Results: The MIC_{50} values for CRO and FEP were 0.19 µg/mL and 0.75 µg/mL, respectively, and the MIC_{90} values were 0.75 µg/mL and 2 µg/mL, respectively. The MIC range was 0.016 to 8 µg/mL and 0.047 to 16 µg/mL for CRO and FEP, respectively. EA between CRO and FEP was only 3% (8/239). CA between CRO and FEP was 79% (188/239). There were no isolates that were CRO nonsusceptible and FEP susceptible. Conversely, 21% (51/239) of the clinical isolates were classified as CRO susceptible and FEP nonsusceptible using the currently established breakpoints.

Conclusions: FEP MIC was higher than that of CRO for *S. mitis/oralis* clinical isolates from blood cultures, with significant discordance in both EA and CA. These data suggest that the FEP breakpoint should not be inferred from the CRO breakpoint of 1 µg/mL for *S. mitis/oralis* inasmuch as the MIC distribution is different for the two drug-organism combinations. Further studies are needed to elucidate the clinical implications of the MIC discordance, but it is likely that the current breakpoints overcall FEP resistance in *S. mitis/oralis* thereby leading to augmented utilization of non-β-lactam antimicrobials and prolonged antimicrobial therapy.

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