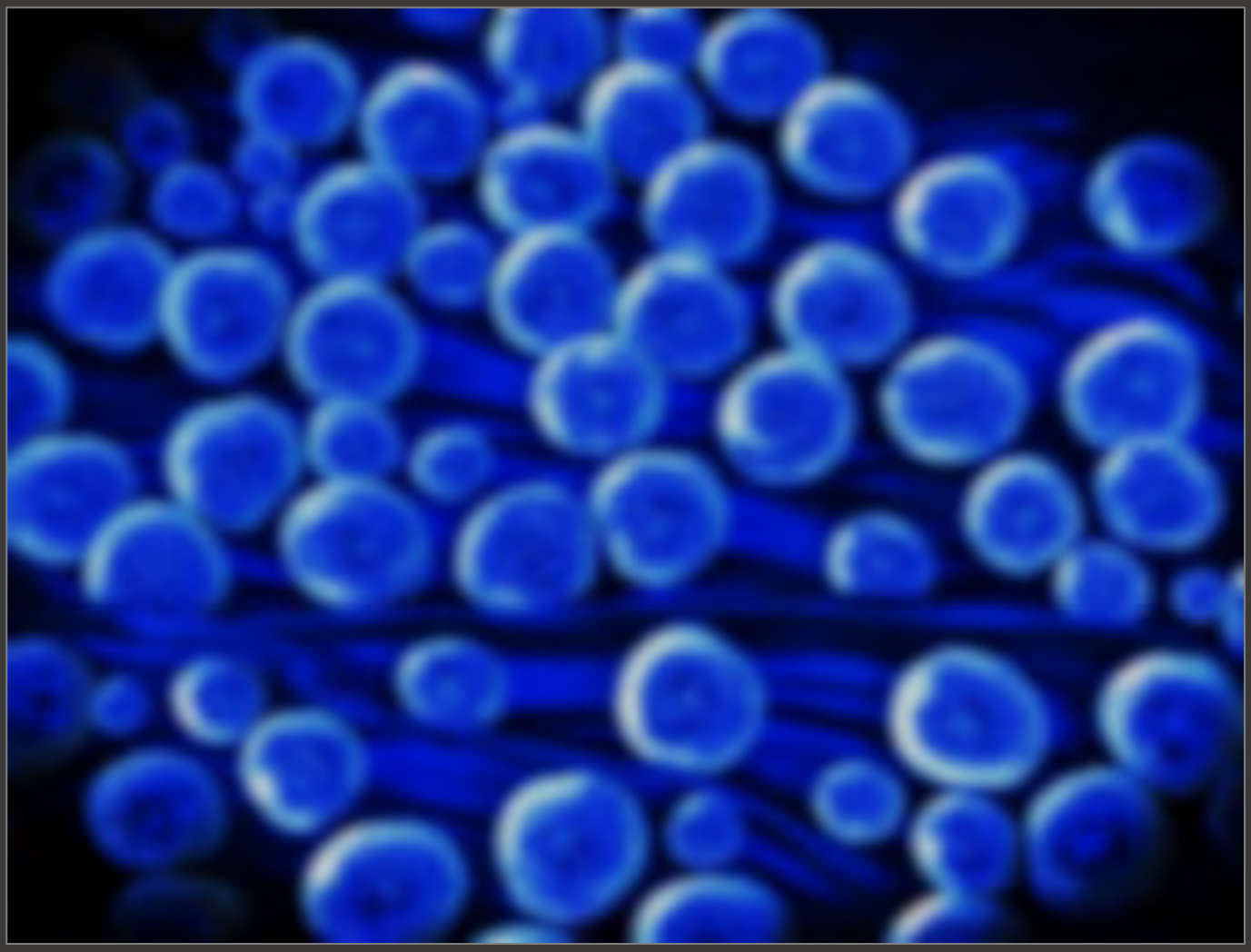
5th Annual Texas Medical Center Antimicrobial Resistance & Stewardship Conference



January 19-21, 2022

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 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 Rresearch, Regenerative Medicine, Single Cell Omics, Theoretical and Computational Neuroscience, Translational Imaging and Translational Pain Research. GCC training programs currently focus on Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Pharmacological Sciences, Precision Environmental Health Sciences and Antimicrobial Resistance. 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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Gulf Coast Consortia - Antimicrobial Resistance Consortium
T32 Training Programs (Texas Medical Center, Emory, UPMC)
NIH Antimicrobial Resistance Leadership Group

5th Annual Texas Medical Center Antimicrobial Resistance & Stewardship Conference
January 19-21, 2022

Day 1 - Wednesday, January 19, 2022
Mechanisms of Resistance and Drug Discovery

7:30 - 8:20 *Career Mentoring: Clinical Scientist Pathway*
Barbara Trautner, MD, PhD
Baylor College of Medicine, Houston, TX
Anthony Flores, MD, PhD
University of Texas Health Science Center, Houston, TX.
Sam Shelburne, MD, PhD
UT MD Anderson Cancer Center, Houston, TX

8:30 - 8:40 *GCC Welcome*
Suzanne Tomlinson, PhD, MBA
Gulf Coast Consortia

8:40 - 8:45 *Welcome*
Cesar A. Arias, MD, PhD
Houston Methodist Hospital and Weill Cornell Medical College

8:45 - 9:10 *ARLG and the Future of Clinical Research in Antimicrobial Resistance*
Vance Fowler, MD, MPH
Duke University, Durham, NC

Session 1
Conveners: **Julian Hurdle, PhD**, Texas A&M Health Science Center
William Miller, MD, Houston Methodist Hospital and Weill Cornell Medical College

9:10 - 9:35 *Effects of Antibiotics on Cell Cycle Progression of Staphylococcus aureus*
Mariana Gomes de Pinho, PhD
University of Lisbon, Lisbon, Portugal

9:35 - 10:00 *The Ribosome as an Antimicrobial Target*
Alexander Mankin, PhD
University of Illinois, Chicago, IL

10:00 - 10:25 *Fluoroquinolones, Topoisomerases, and Supercoiled DNA*
Lynn Zechiedrich, PhD
Baylor College of Medicine, Houston, TX

10:25 - 10:40 Vendor Show: Platinum Vendor
Abbvie Pharmaceuticals Anti-Infectives Introduction
Krista Blevins
Leslie Lindsey
Sean Wells
Paul Bruegger
Dennis Zavidny
Gerard Barber
Abbvie Pharmaceuticals

10:40-10:55 Break

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: **Shantanu Guha, PhD, MPH**
University of Texas Health Science Center at Houston, Houston, TX
Edwin Chen, PhD, University of Pittsburgh, Pittsburgh, PA

10:55 - 11:10 *Discovery of Inhibitors of Antimicrobial Resistance Enzymes Using a Focused DNA-Encoded Library*
Suhyeorn "Jane" Park
Baylor College of Medicine, Houston, TX

11:10 - 11:25 *Exploration of a Synthetic Retinoid Scaffold for the Treatment of Persistent MRSA Infections*
Cassandra Schrank
Emory University, Atlanta, GA

11:25 - 11:40 *Pareto Optimality Fronts Model as a Tool for Understanding Evolutionary Tradeoffs of Antibiotic Resistance*
Francine Arroyo, PhD
University of Pittsburgh, Pittsburgh, PA

Convener: **Lynn Zechiedrich, PhD**
Baylor College of Medicine, Houston, TX

11:40 - 12:20 **Keynote Lecture**
Is Phage Therapy Here to Stay?
Paul Turner, PhD
Yale University, New Haven, CT

12:20 - 2:00 Lunch/Rapid Fire/Poster session

12:20-12:40 Lunch

Convener: **Suzanne Tomlinson, PhD, MBA**
Gulf Coast Consortia

12:40-1:10 **Rapid Fire Presentations**
Saoirse Disney-McKeethen, Rice Univ.
Thomas Horvath, Baylor College of Medicine
Firas Said Midani, Baylor College of Medicine
April Nguyen, University of Texas Health Science Center
Zhizeng Sun, Baylor College of Medicine
Adeline Supandy, Rice University

1:10 -2:00 **Poster Presentations** Posters for each day can be viewed here

Session 3
Conveners: **Tim Palzkill, PhD,** Baylor College of Medicine
Cecilia Tran, PharmD, Houston Methodist Research Institute

2:00 - 2:25 *Carbapenemases in the 21st Century*
Jim Spencer, PhD
University of Bristol, United Kingdom

2:25 - 2:50 *Antibiotic Resistance in Emergent Pathogens*
Ashok Chopra, PhD
University of Texas Medical Branch, Galveston, TX

2:50 - 3:15 *Microfluidic Technology to Study Bacterial Evolution to Antibiotics*
Yousif Shamoo, **Phd**
Rice University, Houston, TX

3:15 - 3:30 Break

Session 4 *NIH Antimicrobial Resistance Leadership Group (ARLG)*
Early Stage Investigators

Conveners: **Anthony Harris, MD MPH**, University of Maryland, Baltimore, MD
Henry Chambers MD, University of California, San Francisco, CA

NIH Antimicrobial Resistance Leadership Group (ARLG) - Early Stage Investigators

3:30 - 3:45 *DOOR Analysis and cUTI*
Jessica Howard-Anderson, MD
Emory University, Atlanta, GA

3:45 - 4:00 *DOOR Endpoint Analysis in Intraabdominal Infection: FDA-NIH Collaboration*
Air Force 2nd Lt. Tori Kinamon
Duke University, Durham, NC

4:00 - 4:15 *DOTS Trial: Dalbavancin as an Option for Treatment of Staphylococcus aureus Bacteremia*
Nicolas Turner, MD
Duke University, Durham, NC

4:15 - 4:30 *PHAGE Trial*

Maria Souli, MD
Duke University, Durham, NC

Session 5 Selected Abstracts

Convener: **Sam Shelburne, MD, PhD**, UT MD Anderson Cancer Center, Houston, TX
Natasha Kirienko, PhD, Rice University, Houston, TX

4:30 - 4:45 *Peptidoglycan Recycling Contributes to Outer Membrane Integrity and Carbapenem Tolerance in Acinetobacter baumannii*
Nowrosh Islam, UT Arlington

4:45 - 5:00 *Anatomy of an Extensively Drug Resistant Klebsiella pneumoniae Outbreak in Tuscany, Italy*
Melissa Martin, **PhD**
Walter Reed Army Institute of Research

5:00 - 5:15 *Combination Eravacycline Therapy for Carbapenem-Resistant Acinetobacter baumannii Pneumonia*
Melissa Jackson, **PharmD**
Parkland Health & Hospital System

Day 2 - Thursday, January 20, 2022
Translational and Clinical Aspects of Antibiotic Resistance

7:30 - 8:20 *Career Mentoring: PharmD Translational/Clinical Scientist Pathway*
Cecilia Tran, PharmD
Houston Methodist Research Institute, Houston, TX
William Musick, PharmD
Houston Methodist Hospital, Houston, TX
Vincent Tam, PharmD,
University of Houston, Houston, TX

Session 6
Conveners: **Blake Hanson, PhD**, University of Texas Health Science Center, Houston, TX
Vincent Tam, PharmD, University of Houston, Houston, TX

8:30 - 8:55 *Combination Therapies for Gram-negative Bacteria*
David van Duin, MD PhD
University of North Carolina, Durham, NC

8:55 - 9:20 *Combination Therapies for Gram-positive Bacteria*
George Sakoulas, MD
University of California, San Diego, CA

9:20 - 9:45 *Combined Genotypic and Phenotypic Antibiotic Susceptibility Testing Through Gene Expression Profiling*
Roby Bhattacharyya, MD PhD
Massachussets General Hospital and Broad Institute, Harvard University, Boston, MA

9:45 - 9:55 Vendor Show: Gold Vendor
Rapid Diagnostics and the Impact on Sepsis
John Sperzel
T2 Biosystems

9:55-10:15 Break

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: **Cassandra Schrank,** Emory University, Atlanta, GA

10:15 - 10:30 *Microbial Therapeutics as Antibiotic Alternative to Suppress Gastrointestinal Pathogen Growth*
Eva Preisner, PhD
Baylor College of Medicine, Houston TX

10:30 - 10:45 *Lost in Translation: Developing Novel Antibiotics Against Bacterial Ribosome Rescue*
Pooja Srinivas
Emory University, Atlanta, GA

10:45 - 11:00 *Development of Novel Antifungals Against Candida Based on an Antifungal Peptide Produced by E. faecalis*
Shantanu Guha, PhD, MPH
University of Texas Health Science Center, Houston, TX

11:00 - 11:15 *Mutations that Inactivate the Tricarboxylic Acid Cycle in Staphylococcus aureus Arise During Persistent MRSA Bacteremia*
Edwin Chen PhD
University of Pittsburgh, Pittsburgh, PA

11:15 - 11:30 *An Epidemiologic Exploration of Vancomycin Resistance in Clostridioides difficile*
Taryn Eubank, PharmD
University of Houston, Houston, TX

11:30 - 12:10 **Keynote Lecture**
Tigers by The Tail: Integrated Analyses of Emerging Infectious Diseases
James Musser, MD, PhD
Houston Methodist Hospital and Weill Cornell Medical College, Houston, TX

12:10 - 2:00 Lunch/Rapid Fire/Poster session
12:10-12:30 Lunch

Convener: **Suzanne Tomlinson, PhD, MBA**
Gulf Coast Consortia

12:30-1:05 **Rapid Fire Presentations**
Lorena Diaz, Universidad El Bosque
Jesus Duran Ramirez, University of Texas Health Science Center
Stephanie Egge, University of Texas Health Science Center
Sara Gomez-Villegas, Houston Methodist Research Institute

Thanh Le, University of Houston
Daniel Stanton, University of Texas Medical Branch
LiyangZhang, Rice University

1:05-2:00 **Poster Presentations** Posters for each day can be viewed here

Session 8
Conveners: **Cesar A. Arias MD, PhD**
Houston Methodist Hospital and Weill Cornell Medical College, Houston, TX

2:00 - 3:00 *Challenging Clinical Cases in Antimicrobial Resistance*
Samuel Shelburne, MD, PhD
UT MD Anderson Cancer Center, Houston, TX
David van Duin, MD PhD
University of North Carolina, Durham, NC
William Miller, MD
Houston Methodist Hospital and Weill Cornell Medical College, Houston, TX

Session 9 ARLG Session 2
Understanding the Enemy: Clinical and Microbiological Characterization of MDR Gram Negative Bacteria

Conveners: **Vance Fowler, MD, MPH**, Duke University, Durham, NC
Helen Boucher MD, Tufts University, Boston, MA

3:00 - 3:15 *Characterization of MDRO in South America*
Jinnethe Reyes, PhD
Universidad El Bosque, Bogota, Colombia

3:15 - 3:30 *SNAP International*
Yohei Doi, MD, PhD
University of Pittsburgh, Pittsburgh, PA

3:30 - 3:45 *POP International*
Mike Satlin, MD
Weill Cornell Medical College, New York, NY

3:45 - 4:00 *SHREC*
David van Duin, MD, PhD
University of North Carolina, Durham, NC

4:00 - 4:30 Break

Session 10
Conveners: **Tor Savidge, PhD**, Baylor College of Medicine, Houston, TX
Todd Treangen, PhD, Rice University, Houston, TX

4:30 - 4:55 *Emerging Fungal Diseases and Resistance*
Mihalis Lionakis, MD
NIAID, Bethesda, MD

4:55 - 5:20 *New Antifungals*

Dimitrios Kontoyannis, MD
UT MD Anderson Cancer Center, Houston, TX

5:20 - 5:45 *An Update on β-lactamase Inhibitors*
Karen Bush, PhD
University of Indiana, Bloomington, IN

Day 3 - Friday, January 21, 2021

Antibiotic Stewardship

7:30 - 8:20 *Careers in Clinical Microbiology*
Micah Bhatti, MD, PhD
UT MD Anderson Cancer Center, Houston, TX
Wesley Long MD, PhD
Houston Methodist Hospital and Weill Cornell Medical College, Houston, TX
Audrey Wanger, PhD
University of Texas Health Science Center, Houston, TX

Session 11
Conveners: **Ed Septimus, MD**
Harvard Medical School and Texas A&M College of Medicine, Houston, TX
Kevin Garey, PharmD
University of Houston, Houston, TX.

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

8:35 - 9:05 **Keynote Lecture**
Dissecting Epidemics of Multidrug-resistant Organisms
Cesar A. Arias, MD, PhD
Houston Methodist Hospital and Weill Cornell Medical College, Houston, TX

9:05 - 9:30 *Pharmacokinetic/Pharmacodynamics of Antibiotics in Renal Replacement Therapies*
Bruce Mueller, PharmD
University of Michigan, Ann Harbor, MI

9:30 - 9:55 *The INSPIRE Trial*
Shruti Gohil, MD, MPH
University of California, Irvine, CA

9:55 - 10:25 Break

Session 12
Conveners: **Kristi Kuper**, **PharmD**
Tabula Rasa HealthCare/DoseMeRx

10:25 - 10:50 *Making the Cut: Antimicrobial Stewardship in Surgery*
Rachel Britt, PharmD
University of Texas Medical Branch, Galveston, TX

10:50 - 11:15 *Impact of the Pandemic on Antimicrobial Resistance*

Priya Nori, MD
Albert Einsten College of Medicine, Bronx, NY

11:15 - 11:40 *An Update on the Management of Clostridiodes difficile Infections*
Kevin Garey, PharmD
University of Houston, Houston, TX

11:40 - 1:30 Lunch/Rapid Fire/Poster session
11:40-12:00 Lunch

Convener: **Suzanne Tomlinson, PhD, MBA**
Gulf Coast Consortia

12:00-12:30 **Rapid Fire Presentations**
Andrew Chou, Michael E DeBakey VA Medical Center/Baylor College of Medicine
Sabrina Green, Baylor College of Medicine
Larissa Grigoryan, Baylor College of Medicine
Lindsey Laytner, Baylor College of Medicine
Timothy Straub, SERES Therapeutics
Marissa Valentine-King, Baylor College of Medicine

12:30 -1:20 **Poster Presentations** Posters for each day can be viewed here

1:20 - 1:50 Panel Discussion: *Nursing Homes Response to COVID-19: Lessons Learned*

Charlene Evans Offiong, PharmD
Houston Health Department, Houston, TX

Oluwayemisi Aikulola, MD, MPH
Houston Health Department

Catherine Anglin
Texas Health and Human Services

Abisola Oladimeji, MD, MPH
Bureau of Epidemiology, Houston Health Department

Tolu Olumuyiwa, MPH
Houston Health Department

Shawn Tupy, MT, MBA
Texas DSHS

Vijisha C Vijayan, BDS, MPH
Houston Health Department

Session 13
Conveners: **Robert Atmar, MD,** Baylor College of Medicine, Houston, TX

1:50 - 2:15 *Antifungal Susceptibility Testing of Molds: When, Why, and How*
Nathan Wiederhold, PharmD

University of Texas San Antonio

2:15 - 2:40 *A Daily Infusion of OPAT Education*
Monica Mahoney, PharmD
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

2:40 - 3:05 *An Update on COVID-19 Vaccines*
Hana El Sahy, MD
Baylor College of Medicine, Houston, TX.

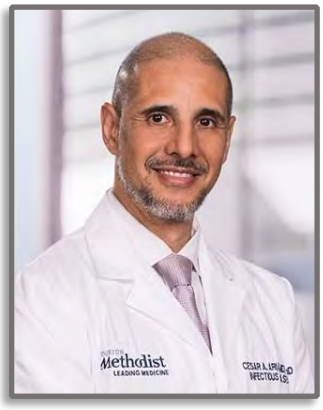
3:05 Closing remarks

Oluwayemisi Aikulola, MD, MPH
Staff Epidemiologist, Division of Disease Prevention and Control

Nursing Homes Response to COVID-19: Lessons Learned

Dr. Oluwayemisi Aikulola is an Epidemiologist Specialist who currently serves as the City of Houston Health Department's (HHD) focal person to Nursing Homes in Houston. In this role, she supports the early detection and containment of COVID-19 infection among residents/staff of nursing homes conducting facility level field investigations and providing onsite COVID-19 infection prevention and control guidance to over 40 LTCFs within the City of Houston. She also provides technical guidance to epidemiologists on the HHD Nursing Home team in managing COVID-19 outbreaks and implementing infection control protocols.

With her background and experience in clinical medicine, she has harnessed her expertise in analyzing the trends of COVID-19 vaccine breakthrough/re-infection cases, responding to complex reports and inquiries on COVID-19 cases/clusters and interpreting COVID-19 preventive measures to support nursing homes' leadership and staff (executive administrators, facility managers, directors of nursing etc.) based on the Centers for Disease Control and Prevention (CDC) and HHD guidelines. She holds a Master of Public Health Degree from the George Washington University and a Bachelor of Medicine and Surgery degree from Obafemi Awolowo University, Nigeria.



Cesar A. Arias, MD, MSc, PhD
Co-director
Center for Infectious Diseases Research

Dissecting Epidemics of Multidrug-resistant Organisms
Keynote Lecture

Cesar A. Arias, MD, MSc, PhD. is the Chief, Division of Infectious Diseases at Houston Methodist Hospital. He is Professor and John F. III and Ann H. Bookout Distinguished Chair and Co-Director Center for Infectious Diseases Research, Houston Methodist Research Institute and Weill Cornell Medical College.

Dr. Arias is a nationally and internationally recognized physician-scientist conducting NIH-funded basic, translational and clinical research on mechanisms and molecular epidemiology of antibiotic resistance. 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. Dr Arias was a standing member of the NIH/NIAID Microbiology and Infectious Diseases study section since 2012 and has served a reviewer for the European Union Joint Program Initiative for Antimicrobial Resistance (JPIAMR). 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 elected Fellow of the Infectious Diseases Society of America in 2015 and inducted to the American Society for Clinical Investigation in 2015 and American Academy of Microbiology in 2019. He serves as Editor-In-Chief of Antimicrobial Agents and Chemotherapy. Dr Arias also serves as Chair of the Gulf Coast Consortium on Antimicrobial Resistance in Houston (a partnership between 7 institutions in the Texas Medical Center) and is part of World Health Organization Antibiotic Pipeline Panel and the NIH-funded Antimicrobial Resistance Leadership Group. Dr Arias founded the Molecular Genetics and Antimicrobial Resistance Unit and International Center for Microbial Genomics at Universidad El Bosque, Bogota, Colombia.

Francine Arroyo, PhD
Postdoctoral Fellow

Pareto Optimality Fronts Model as a Tool for Understanding Evolutionary Tradeoffs of Antibiotic Resistance

Dr. Arroyo studies how bacterial lifestyles 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 geothermal hot springs under the mentorship of Dr. Patricia Siering. She was awarded a PhD in Microbiology from Cornell University in the lab of Dr. 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. Dr. Arroyo is currently in the lab of Dr. Vaughn Cooper at the University of Pittsburgh School of Medicine where she investigates 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. Ongoing work includes exploring the evolutionary tradeoff between phage resistance and antibiotic susceptibility in Acinetobacter biofilms.

University of Pittsburgh

Roby Bhattacharyya, PhD
Assistant Professor
Medicine

Combined Genotypic and Phenotypic Antibiotic Susceptibility Testing Through Gene Expression Profiling

Roby Bhattacharyya is an Assistant Professor of Medicine in the Infectious Diseases Division at Massachusetts General Hospital and Harvard Medical School and an Associate Member at the Broad Institute in the Infectious Disease and Microbiome Program. He is a practicing infectious disease physician at MGH with particular clinical interest in antibiotic-resistant infections and COVID-19. He also leads a research laboratory at the Broad focusing on antimicrobial resistance, transcriptional profiling of pathogens and patients, and rapid diagnostics. Roby grew up in the Chicago area, received his MD and PhD in Biochemistry and Molecular Biology from the University of California at San Francisco, and did Internal Medicine residency, chief residency, and Infectious Disease fellowship training at MGH.

Rachel Britt, PharmD, BCIDP
Clinical Practice Specialist
Infectious Diseases

Making the Cut: Antimicrobial Stewardship in Surgery

Dr. Rachel Britt is a Pharmacy Clinical Practice Specialist in Infectious Diseases at UTMB Health in Galveston, Texas. She received her PharmD from The University of Texas at Austin College of Pharmacy and completed her PGY1 Pharmacy Practice and PGY2 Infectious Diseases residencies at Beth Israel Deaconess Medical Center in Boston, Massachusetts.

Dr. Britt's practice interests include antimicrobial stewardship, gram-negative resistance, precepting, and mentorship. She actively serves the profession through involvement in professional organizations and is currently serving as Chair of the Society of Infectious Diseases Pharmacists Publications and Podcasts Committee Chair and Chair of the American College of Clinical Pharmacy Infectious Diseases Practice and Research Network Networking Committee.

Karen Bush, PhD
Professor of Practice & Interim Director,
Biotechnology Program

An Update on β-lactamase Inhibitors

Karen Bush is currently a Professor of Practice and interim Director of the Biotechnology Program at Indiana University. Prior to moving to IU in 2009, she spent 35 years on antibiotic discovery/development teams in the pharmaceutical sector. Her main research focus has been the study of beta-lactamases. Her lab provided the biochemical characterization of some of the first ESBLs in the USA, the second plasmid-encoded AmpC cephalosporinase and the first KPC carbapenemases. Together with George Jacoby and Antone Medeiros, she established a widely-recognized functional nomenclature for beta-lactamases. At Squibb, Lederle, and Johnson & Johnson, she worked on teams that were involved with the discovery and/or development of aztreonam, piperacillin-tazobactam, levofloxacin, ceftobiprole and doripenem. She was the drug discovery team leader or team member associated with the entry of 9 Investigational Drugs into Phase 1 clinical trials.

Her lab has recently been tracking carbapenem resistance in Gram-negative bacteria in central Indiana, and has tested investigational antibiotics against these pathogens. She has served as a consultant for more than a dozen biotech companies, has been a reviewer for NIH, IMI, CARB-X and other granting agencies, and was a scientific advisor for the TB Alliance, the Pew Charitable Trust, GARDP, and CARB-X. She has published 225 peer-reviewed publications. In addition to serving on the ICAAC and ASM Microbe Program Committees for a total of nine years, she was an Editor or Associate Editor for Antimicrobial Agents and Chemotherapy, mBio, EcoSal Plus and Antimicrobial Therapeutics Reviews. She received the E. P. Abraham Award for Beta-Lactamase Research in 2007, the CLSI Excellence in Standards Development Award in 2015 and was the first female to receive the ISC Hamao Umezawa Memorial Award in 2017. In 2000 she was elected a Fellow of the American Academy of Microbiology and was named an ISAC Fellow in 2019 by the International Society of Antimicrobial Chemotherapy.

Edwin Chen MD, PhD
Infectious Diseases Fellow

Mutations that Inactivate the Tricarboxylic Acid Cycle in Staphylococcus aureus Arise During Persistent MRSA Bacteremia

Edwin Chen obtained his MD and PhD at Washington University in St. Louis where he completed his PhD thesis in the laboratory of Niraj Tolia characterizing inhibitory antibodies targeting P. falciparum and P. vivax invasion proteins. He then completed both an Internal Medicine residency and an Adult Infectious Diseases fellowship at the University of Pittsburgh Medical Center. He is currently at T32 post-doctoral scholar at the University of Pittsburgh in the laboratory of Matthew Culyba studying the mechanisms of antibiotic tolerance in persistent S. aureus bacteremia.

Ashok K. Chopra, PhD, CSc
Professor and John S. Dunn Distinguished Chair in Global Health
Dr. Leon Bromberg Distinguished Teaching Professor

Antibiotic Resistance in Emergent Pathogens

Dr. Chopra's research interests during the past two decades have been on identifying virulence factors/mechanisms from several Gram-negative bacteria and to demonstrate their role in causing human diseases. Specifically, his group has focused on type 2-, -3, and -6 secretion systems and quorum sensing, and they have used molecular/genomics/proteomics tools to better understand mechanisms of action of the selected virulence factors. Their emphasis is on gastrointestinal and respiratory diseases as well as necrotizing fasciitis with a focus on bacterial-host cell interactions. In addition, they are developing and testing new vaccines and therapeutics against Tier-1 select agents, such as Yersinia pestis, the causative agent of plague. They are testing new platforms to display antigens from biodefense-related pathogens that are protective. His laboratory has made a startling discovery by characterizing a novel host regulatory molecule which acts as a double-edged sword, being involved in modulating inflammation and playing a pivotal role in causing neurodegenerative disorders, in humans. In addition, they are examining new therapeutics other than antibiotics that could be used to combat diseases caused by antibiotic-resistant bacteria and their potential effects on microbiota. Finally, they have developed and currently testing second generation COVID-19 vaccines in various animal models and evaluating mucosal, humoral, and cellular immune responses. His research has been currently and previously funded through NIH, NSF. American Heart Association, Crohns and Colitis Foundation, Department of Defense, Dunn Foundation, and the private sector.He serves as the Program Director of NIH/NIAID T32 training grant for predocs titled "Biodefense Training Program."

Hana El Sahly, MD
Professor
Molecular Virology and Microbiology

An Update on COVID-19 Vaccines

Dr. Hana El Sahly is a Professor of Molecular Virology and Microbiology at Baylor College of Medicine. She is the Principal Investigator of the NIH funded Vaccine and Treatment Evaluation Unit, and chair of the FDA's Vaccine and Related Biological Product Advisory committee. She is an Infectious Diseases attending at Ben Taub Hospital and her research focuses on clinical vaccine development.

Taryn Eubank, PharmD, BCIDP
Infectious Diseases Pharmacy Clinical Specialist

An Epidemiologic Exploration of Vancomycin Resistance in Clostridioides difficile

Taryn completed her Doctorate in Pharmacy at Harding University in Searcy, Arkansas. She then went on to complete PGY1 and PGY2 in Infectious Diseases residencies at Houston Methodist Hospital in the Texas Medical Center. She is currently completing a postdoctoral fellowship in a T32 Training Program in Antimicrobial Resistance funded by the National Institute of Allergy and Infectious Diseases (NIAID). Her areas of interest include 1) antimicrobial resistance and genetic mechanisms, 2) gastrointestinal microbiome, and 3) translational research.

Vance Fowler, MD, MHS
Professor
Medicine and Molecular Genetics & Microbiology

ARLG and the Future of Clinical Research in Antimicrobial Resistance

Vance Fowler, MD, MHS, Professor, Departments of Medicine and Molecular Genetics & Microbiology, Duke University Medical Center. Dr. Fowler 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 is the Communicating PI of the Antibacterial Resistance Leadership Group. He has over 250 peer-reviewed publications with > 19,000 citations.

Kevin W. Garey, PharmD, MS
Professor and Chair
An Update on the Management of Clostridiodes difficile Infections

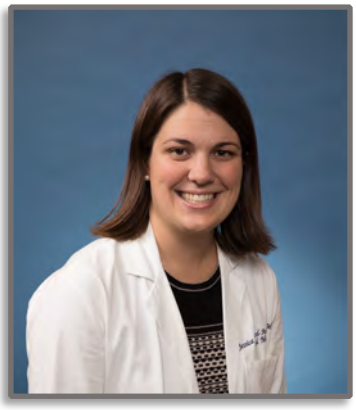
Kevin Garey, PharmD, MS, FASHP is a Professor at the University of Houston College of Pharmacy and Chair of the Department of Pharmacy Practice and Translational Research. He is an Adjunct Professor at the University of Texas School of Public Health and a Clinical Specialist and Researcher at Baylor St. Luke's Medical Center, Houston, Texas. He received a Bachelor of Science in Pharmacy degree from Dalhousie University in Halifax, Nova Scotia, Canada, a Doctor of Pharmacy from SUNY Buffalo in Buffalo, NY, and a Masters of Science in Biometry from the University of Texas School of Public Health. Postdoctoral training includes a pharmacy practice residency at Bassett Healthcare, Cooperstown, NY and infectious disease specialty residency and fellowship training at the University of Illinois at Chicago, Chicago, IL.

Dr. Garey is a Fellow of the Infectious Diseases Society of American (IDSA) and a member of the IDSA-Society of Healthcare Epidemiology of America (SHEA) practice guidelines for C. difficile infection. He is an active member of the Society of Infectious Diseases Pharmacists (SIDP) and a Fellow of the American Society of Health-system Pharmacists (FASHP) and American College of Clinical Pharmacy (FCCP). He has been awarded several national awards including the ASHP Best Practice Award in Health-system Pharmacy Administration (2010), the ASHP Drug Therapy Research Award (2007), and the SIDP Impact Paper in Infectious Diseases Pharmacotherapy Research Award (2007, 2012). Dr. Garey's research is supported by the National Institute of Health, the Centers for Disease Control and Prevention, and the biotechnology industry. He has published over 230 manuscripts centered on clinical and translational research in healthcare associated infections including healthcare-related infections, candidemia, and Clostridium difficile infection.

Shantanu Guha, PhD, MPH
Postdoctoral Fellow

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

Shantanu Guha attended Austin College in Sherman, TX, receiving a Bachelor's (BA) degree in Biology in 2011. It was during this time at Austin College that he first developed an interest in scientific research, studying the misfolding patterns of Beta-2-microglobulin of the MHC Class I molecule. Directly following college, Shantanu moved to New Orleans, LA to join Tulane University to earn a Master of Public Health (MPH) degree in Epidemiology, which was received in 2013. During this time, his interest in pathogen-based research began as he studied the development of novel drug targets against the malaria parasite, Plasmodium falciparum, After a one-year hiatus from graduate school to gain laboratory experience, Shantanu started the Graduate Program in Biomedical Sciences at Tulane University in the fall of 2015 and earned his Ph.D in 2020 studying and identifying a novel peptide-based virulence factor of Ebola virus. Following graduation, Shantanu started a postdoctoral research fellowship at the University of Texas Health Sciences Center in Houston at McGovern Medical School, where he now works on the preclinical development of novel peptide antifungal agents.



Jessica Howard-Anderson, MD, MSc
Assistant Professor
Division of Infectious Diseases
DOOR Analysis and cUTI

Dr. Howard-Anderson is an Assistant Professor of Medicine at Emory University School of Medicine and the Associate Hospital Epidemiologist at Emory University Hospital Midtown. She completed her medical school, residency and chief residency at University of California Los Angeles. Dr. Howard-Anderson completed fellowship training in infectious diseases at Emory University in 2021 and during her fellowship obtained a Master of Science in Clinical Research and was selected for a research fellowship with the Antibacterial Resistance Leadership Group. Dr. Howard-Anderson's research focuses on: 1) epidemiology and risk factors for multidrug-resistant gram-negative infections, 2) using novel methodologies such as DOOR (desirability of outcome ranking) to better understand the clinical outcomes of patients with multidrug-resistant organisms, and 3) employing diagnostic stewardship to improve antimicrobial prescribing and decrease healthcare associated infections.

Nowrosh Islam
Graduate Student
Biology

Peptidoglycan Recycling Contributes to Outer Membrane Integrity and Carbapenem Tolerance in Acinetobacter baumannii

Noweosh Islam is a 3rd year Ph.D. Student, working on antibiotic tolerance under the supervision of Dr. Joseph Boll. She is an enthusiastic and dedicated microbiologist and believes working with the microbes is fun because they are smarter than humans!

Besides lab, she likes roaming around different places, shopping, music and spending time with her friends and family.

Melissa Jackson, PharmD
PGY-2 Infectious Diseases Pharmacy Resident

Combination Eravacycline Therapy for Carbapenem-Resistant Acinetobacter baumannii Pneumonia

Melissa Jackson is currently a PGY-2 Infectious Diseases Pharmacy Resident at Parkland Health and Hospital System. She completed her BS in Chemistry and her Doctor of Pharmacy at the University of Texas at Austin. During her time in pharmacy school she was the vice president of the American Society of Health System Pharmacists student chapter at UT and member of The Rho-Chi honor society. After graduation, she plans to become a board-certified infectious diseases pharmacist.

Air Force 2nd Lt. Tori Kinamon
Graduate Student

DOOR endpoint analysis in Intraabdominal infection: FDA-NIH Collaboration

Air Force 2nd Lt. Tori Kinamon is an MD Candidate at Duke University School of Medicine and the recipient of the FDA Antibacterial Drug Resistance (DOOR) Fellowship. Her research focuses on evaluating and developing ordinal endpoints using the Desirability of Outcome Ranking (DOOR) approach for anti-infective clinical trials with the aim of assessing global benefits and risks of antibacterial intervention. She is a member of the Antibacterial Resistance Leadership Group DOOR Exploratory Task Force. Her clinical and research interests include antibacterial resistance and staphylococcal infections.

Dimitrios P. Kontoyiannis, MD, ScD, PhD (Hon), FACP, FIDSA, FECMM, FAAM, FAAAS
Robert C Hickey Chair in Clinical Care
Deputy Head, Division of Internal Medicine

New Antifungals

Dimitrios P. Kontoyiannis is Professor and the Robert C Hickey Chair in Clinical Care and Deputy Head, Division of Internal Medicine at the MD Anderson Cancer Center. He is considered the leading clinical mycology expert world-wide (expertscape.com) and listed in the 1% of most highly cited and influential researchers world-wide (Web of Science Group) in clinical, translational and experimental mycology.

University of Texas MD Anderson Cancer Center

Mihalis Lionakis, MD
Head of the Fungal Pathogenesis Section
Emerging Fungal Diseases and Resistance

Dr. Lionakis is a physician-scientist and Head of the Fungal Pathogenesis Section in NIAID's Laboratory of Clinical Immunology and Microbiology where he is Deputy Chief. He obtained his MD and ScD from the University of Crete, Greece. He did postdoctoral research training at MD Anderson Cancer Center, followed by Internal Medicine Residency at Baylor College of Medicine, and Infectious Disease Fellowship at NIAID/NIH. He established his own laboratory in 2012 at NIAID and received tenure in 2017. Dr. Lionakis' laboratory bench-to-bedside research focuses on 1) better understanding the genetic and immune defects that underlie susceptibility to fungal infections in humans and on 2) cellular and molecular factors that regulate the immune response against fungi in clinically relevant animal models. His work has defined precise genetic, biochemical, immunologic, and cellular disease mechanisms that have led to targeted immunotherapies. Dr. Lionakis has published >180 peer-reviewed papers in journals such as Science, Science Translational Medicine, Science Immunology, Nature Immunology, JCI, Journal of Experimental Medicine, Cell Host Microbe, Cancer Cell, and others. He is a Member of the American Society for Clinical Investigation, a Fellow of the American Academy of Microbiology, and a Fellow of the Infectious Diseases Society of America. He has received several awards including the NIH Director's award, the IDSA Oswald Avery Award for Early Achievement, the Junior Investigator Award from the Immunocompromised Host Society, and the American College of Physicians Walter J. McDonald Award for Early Career Physicians.

Monica V. Mahoney, PharmD, BCPS, BCIDP, FCCP
Clinical Pharmacy Specialist, Infectious Diseases
A Daily Infusion of OPAT Education

Dr. Monica Mahoney is a graduate of the Massachusetts College of Pharmacy and Health Sciences. She completed PGY1 and PGY2 training in infectious diseases at Tufts Medical Center. For 12 years, she has worked at the Beth Israel Deaconess Medical Center, the past 2 years specifically in the outpatient ID and OPAT clinics. She is involved in a number of national organizations, including SIDP, the society of infectious diseases pharmacists and IDSA. You can find her tweeting way too much, under the Twitter handle @mmPharmD.

Beth Israel Deaconess Medical Center, Harvard Medical School

Alexander Mankin, PhD
Professor
Pharmaceutical Sciences

The Ribosome as an Antimicrobial Target

Alexander Mankin received his PhD (1982) and D.Sci (1989) degrees from Moscow State University, Russia. He joined the faculty of the University of Illinois at Chicago in 1993 and currently holds the position of Distinguished University Professor and A. Neyfakh Collegiate Professor in the Department of Pharmaceutical Sciences and Center for Biomolecular Sciences. In 2014, Mankin was elected a Fellow of the American Academy of Microbiology. He has received the ICAAC Program Committee Award (2006), UIC Researcher of the Year Award (2008), Outstanding Service Recognition Award of the American Scoiety for Microbiology (2011), Paul R. Dawson Biotechnology Award from the American Association of the Colleges of Pharmacy (2013) among others. Mankin, together with Prof Vázquez-Laslop, co-head their lab that studies the functions of the ribosome in protein synthesis, principles of antibiotic action and molecular mechanisms of resistance.

University of Illinois at Chicago

Melissa Martin, PhD
Senior Research Scientist

Anatomy of an Extensively Drug Resistant Klebsiella pneumoniae Outbreak in Tuscany, Italy

Melissa Martin completed her PhD in Microbiology from the London School of Hygiene and Tropical Medicine followed by a post-doctoral position in the laboratory of Professor Michael Gilmore at Harvard Medical School and Massachusetts Eye and Ear Infirmary. Melissa joined the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) at the Walter Reed Army Institute of Research in November of 2020 as a Senior Research Scientist. Her research focuses on surveillance of MDR organisms and the use of genomic sequencing and phylogenetics to study the evolution and transmission of MDR bacterial pathogens.

William R. Miller, MD
Assistant Professor
Infectious Diseases
Infectious Diseases

William R. Miller, M.D. is an Assistant Professor with the Division of Infectious Diseases at Houston Methodist Hospital and member of the Center for Infectious Diseases Research at the Houston Methodist Research Institute. 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.

Bruce A. Mueller, PharmD, FCCP, FASN, FNKF
Interim Dean and Professor of Clinical Pharmacy

Pharmacokinetic/Pharmacodynamics of Antibiotics in Renal Replacement Therapies

Bruce Mueller is Interim Dean and Professor of Clinical Pharmacy at the University of Michigan College of Pharmacy. Dean Mueller received his BS in Pharmacy from the University of Wisconsin and PharmD and residency training at the University of Texas. During his academic career, he has led a multidisciplinary clinical and laboratory research focusing on nutrition and pharmacotherapy in patients with kidney failure. He has published over 145 peer-reviewed scientific papers focused on how to treat patients receiving dialysis therapies in the inpatient and outpatient settings. He and his teams of colleagues and trainees have led dozens of trials investigating new and existing drugs with the primary purpose of finding optimal doses for drugs removed by dialysis therapies.

In recognition for his decades of mentoring professional and graduate students, as well as post-doctoral fellows in his laboratory, he received the 2015 University of Michigan Institute for Clinical & Health Research Distinguished Clinical and Translational Research Mentor Award. In 2021, he was awarded the University of Michigan Center for Interprofessional Education Distinguished Leadership Award. In recognition for his contributions, Dr. Mueller has been named a Fellow of the American College of Clinical Pharmacy, American Society of Nephrology, and the National Kidney Foundation.

James M. Musser, MD, PhD
Fondren Distinguished Presidential Endowed Chair and Chair of the Department of Pathology and Genomic Medicine

Tigers by The Tail: Integrated Analyses of Emerging Infectious Diseases

Keynote Lecture

James M. Musser, M.D., Ph.D. holds the Fondren Distinguished Presidential Endowed Chair and is Chair of the Department of Pathology and Genomic Medicine for the Houston Methodist Hospital System. He also directs the Center for Infectious Diseases and the Laboratory of Clinical and Translational Human Infectious Diseases Research at the Houston Methodist Research Institute. He is Professor of Pathology and Laboratory Medicine, Weill Cornell Medical College.

Dr. Musser earned his M.D. and Ph.D. degrees from the University of Rochester School of Medicine. He trained at the Hospital of the University of Pennsylvania and joined the Department of Pathology of Houston Methodist Hospital in 1991. In 1999, he accepted a position with Dr. Anthony Fauci at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, where he was Founding Chief of the Laboratory of Human Bacterial Pathogenesis. In 2005, he and his laboratory moved to Houston Methodist Hospital and Research Institute. He works primarily on group A Streptococcus (the “flesh-eating” pathogen), with a special emphasis on human-pathogen molecular interactions using genome-wide investigative strategies. His group also uses genome-scale analyses to decipher the molecular events underpinning pandemics and pathogen-host interactions. He has had a decades-long interest in developing a group A Streptococcus vaccine. Clinically, his efforts focus on diagnostic microbiology, patient safety, error reduction, and antimicrobial agent resistance.

Dr. Musser is an elected member of many professional societies, including the American Academy of Microbiology, Association of University Pathologists (Pluto Society), American Society for Clinical Investigation, Association of American Physicians, and the American Clinical and Climatological Association. He has been fortunate to receive many national honors and awards, including the ICAAC Young Investigator Award (1992), and the Warner-Lambert/Parke-Davis Award (1999) and Chugai Award for Excellence in Mentoring and Scholarship (2007), both sponsored by the American Society for Investigative Pathology. He received the prestigious Rous-Whipple Award in 2017, sponsored by the American Society for Investigative

Pathology. He is past president of the American Society for Investigative Pathology, served on the Board of Directors, Federation of American Societies for Experimental Biology (FASEB), and was President of FASEB (2018-2019).

He has published more than 450 papers and book chapters in the field of bacterial pathogenesis, bacterial population genetics, and infectious diseases, and has an h-index of 107.

Priya Nori, PhD
Associate Professor
Medicine and Orthopedic Surgery

Impact of the Pandemic on Antimicrobial Resistance

Dr. Priya Nori is an Associate Professor of Medicine and Orthopedic Surgery at the Albert Einstein College of Medicine. She serves as Medical Director of the Antimicrobial Stewardship Program (ASP) and outpatient parenteral antibiotic therapy program (OPAT) at Montefiore Health System and is Program Director of the Infectious Diseases (ID) fellowship program.

She leads Stewardship, Infection Prevention, and ID educational initiatives throughout the medical center. Her academic focus is educational curricula and decision-support tools for stewardship and infection prevention, orthopedic infections, and novel diagnostic technology for rapid microbe identification, and pandemic stewardship. She serves on the Infectious Diseases Society of America's (IDSA) Subcommittee for Antimicrobial Stewardship Curricula, the Centers for Disease Control and Prevention/Society for Healthcare Epidemiology of America's (CDC/SHEA) Outbreak Response Training Program Education Committee, the SHEA Antimicrobial Stewardship Committee, and co-chairs the SHEA stewardship certificate track, 2019-2022. Under her leadership, Montefiore's ASP was awarded the IDSA's Stewardship Center of Excellence distinction in 2018. She is also course director of IDSNY's fellows' course on stewardship, infection prevention, and hospital epidemiology. She was awarded ID fellowship "teacher of the year" in 2015, the Department of Medicine "rising star" award in 2017, and the "Sharon Silbiger Subspecialist Teacher of the Year" award in 2020. She directs the Montefiore COVID-19 Monoclonal Antibody Treatment Program since December 2020.

Charlene Evans Offiong, PharmD
Healthcare Associated Infection Coordinator

Panel Discussion: Nursing Homes Response to COVID-19: Lessons Learned

Charlene Offiong, RPH, PharmD is currently the Division Manager/Healthcare Associated Infection Coordinator for the Houston Health Department (HHD). She has spent the last 5 years growing HHD's HAI program managing several projects such as the Antibiotic Resistant Laboratory Network, Antibiotic Stewardship Collaboratives as well as responding to multi drug resistant organisms (MDROs) investigations. Last year during the start of the 2020 pandemic, she served as team lead for the COVID NH Team response. Her career has spanned various areas of pharmacy practice including academia, clinical psychiatric pharmacy, and pharmaceutical industry. Her past experiences from The Methodist Hospital, Texas Southern University, GlaxoSmithKline, and Novartis has afforded her the ability to be an educator, clinician, and strong proponent of public health.

Abisola Oladimeji, MD, MPH
Division Manager/Medical Congregate Coordinator,
Bureau of Epidemiology|Division of Disease Prevention and Control

Nursing Homes Response to COVID-19: Lessons Learned

Dr. Oladimeji is a public health physician and epidemiologist. Her area of specialty includes public health program management and coordination, emergency preparedness and response. She is vast in developing and evaluating surveillance and early warning systems, epidemiologic research methods, public health workforce capacity development, community engagement and partnerships. She has served on several CDC-funded programs, population studies, and, investigated numerous infectious disease outbreaks both locally and internationally - Ebola outbreak in Africa been one of them. She serves as a Division Manager under the Bureau of Epidemiology at Houston Health Department where she oversees congregate settings. She coordinated the COVID-19 outbreak response in nursing home facilities between 2020 and 2021. She is presently overseeing the program that keeps schools open for in-person learning through COVID-19 testing and implementation of layered-protection. Dr. Abby loves giving voice to data.

Tolu Olumuyiwa, MPH
Public Health Analyst/Infectious Disease Epidemiologist

Nursing Homes Response to COVID-19: Lessons Learned

Tolu Olumuyiwa, MPH has 12+ years of experience in public health. Her previous roles have spanned from quality improvement and public health accreditation, to managing the syndromic surveillance team at the Houston Health Department (HHD) and coordinating prevention and stewardship activities for HHD. She led the prevention and response team for Long Term Care Facilities (LTCF) during the first year of the COVID-19 pandemic. She currently manages the health department's CDC COVID-19 grants portfolio.

Suhyeorn Park
Graduate Student
Pharmacology and Chemical Biology

Discovery of Inhibitors of Antimicrobial Resistance Enzymes Using a Focused DNA-Encoded Library

Suhyeorn Park got her Bachelors of Science in Chemistry and Bachelors of Arts in French Studies from Rice University. She is currently a graduate student in Dr. Timothy Palzkill lab in the Department of Pharmacology and Chemical Biology at Baylor College of Medicine. She was a T32 MBID trainee in 2019-2021. Her research primarily focuses on discovering inhibitors for antimicrobial resistance enzymes using DNA-encoded library platform.

Mariana Pinho, PhD
Associate Professor

Effects of Antibiotics on Cell Cycle Progression of Staphylococcus aureus

Mariana Pinho has studied Chemistry before doing a PhD in Microbiology at the Rockefeller University, New York, followed by a Post-doc on bacterial cell biology, at the University of Oxford, UK. In 2006 she started her research group at the Institute of Technology, Chemistry and Biology from Universidade Nova de Lisboa, Portugal. She is an elected member of the European Molecular Biology Organization (EMBO) and of the European Academy of Microbiology (EAM). Research in the Pinho laboratory aims to understand the complex organization of bacterial cells, focusing on the temporal and spatial regulation of bacterial cell division and morphogenesis, as well as to integrate this information for a better understanding of antibiotic resistance mechanisms in the gram-positive pathogen Staphylococcus aureus.

University of Lisbon

Eva Preisner, PhD
Postdoctoral Researcher
Microbial Therapeutics as Antibiotic Alternative to Suppress Gastrointestinal Pathogen Growth

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.

Jinnethe Reyes, MSc, PhD
Professor Titular

Characterization of MDRO in South America

Jinnethe Reyes, Microbiologist with a PhD in Biological Sciences. Director of the Molecular Genetics and Antimicrobial Resistance Unit of Universidad El Bosque in Bogota, Colombia. she possess a broad experience as a microbiologist and have background in bacterial genetics and surveillance projects coordination with emphasis in Gram positive bacteria like Staphylococcus aureus and Enterococci. She has obtained and made countless contributions to the study of bacterial resistance by multicentric surveillance involving hospitals in Colombia and Latin America. In addition, she participates as a principal investigator from South America in a worldwide project on multidrug resistance organisms for the study of the genomic epidemiology of Enterobacterales resistant to carbapenems, multiresistant Pseudomonas and Acinetobacter.

George Sakoulas, MD
Associate Adjunct Professor
Pediatrics

Combination Therapies for Gram-positive Bacteria

Dr. George Sakoulas is an active infectious disease clinician in the Sharp Healthcare System, San Diego, CA, and an Adjunct Professor in the Division of Host-ˇ Microbe Systems and Therapeutics, Center for Immunity, Infection and Inflammation at the University of California San Diego School of Medicine. Dr. Sakoulas is also an investigator in the UCSD Health Sciences interdisciplinary research and educational initiative called the “Collaborative to Halt Antibiotic-ˇResistant Microbes” or CHARM.

Dr. Sakoulas received his undergraduate degree from Columbia University and his medical degree from Harvard Medical School. He completed his clinical training in internal medicine and infectious diseases at the Beth Israel Deaconess Medical Center in Boston.

Through studying the relationships between antimicrobial resistance and bacterial virulence and the interactions between innate host defense and administered antibiotics, Dr. Sakoulas is working towards developing more efficient and effective therapies, utilizing currently available drugs, for serious infections that are refractory to standard treatments. This includes the repurposing of medications used for other diseases.

Michael Satlin, MD
Infectious Diseases Physician and Associate Professor
POP International

Dr. Michael Satlin an infectious diseases physician and Associate Professor of Medicine and of Pathology and Laboratory Medicine at Weill Cornell Medicine. He is the Clinical Director of the Transplantation-Oncology Infectious Diseases Program at Weill Cornell. He completed medical school at the University of Virginia School of Medicine and internal medicine residency and infectious diseases fellowship training at NewYork-Presbyterian Hospital/Weill Cornell. He also has a Master's Degree in Clinical and Translational Investigation at Weill Cornell. His research focuses on the epidemiology, prevention, and treatment of multidrug-resistant Gram-negative infections, with a focus on immunocompromised hosts, and he has multiple NIH-funded grants to support this work. He is Associate Editor of Journal of Antimicrobial Chemotherapy-Antimicrobial Resistance, serves on the Editorial Board of Journal of Clinical Microbiology, and on the Editorial Advisory Board of Clinical Infectious Diseases. He has authored or co-authored over 85 peer-reviewed manuscripts. He is a Member of the Clinical and Laboratory Standards Institute's (CLSI) Subcommittee on Antimicrobial Susceptibility Testing and Co-Chair of its Breakpoint Working Group. He also serves on the ASM Microbe Program Committee and is a member of multiple committees for the NIAID's Antibacterial Resistance Leadership Group.

Cassandra Schrank
Graduate Student

Exploration of a Synthetic Retinoid Scaffold for the Treatment of Persistent MRSA Infections

Cassandra Schrank earned her B.S. in Chemistry (Xavier University, 2012) and completed her undergraduate thesis of the total synthesis of (+/–)-pilosinine under the advisement of Prof. Richard M. Mullins. Prior to attending graduate school, she spent two years working as a technical associate at Milliken and Co. in Spartanburg, SC as part of the plastic additives research division. She then began graduate school at Emory University in 2018. Currently, she is a fourth year Ph.D. candidate (expected graduation date Spring 2023) under the advisement of Prof. William M. Wuest. Outside of the lab, she enjoys hiking and spending time with her dog and two cats.

Yousif Shamoo, PhD
Vice Provost for Research
Microfluidic Technology to Study Bacterial Evolution to Antibiotics

Yousif Shamoo, Ph.D., has served as the Vice Provost for Research at Rice University since 2014. He was appointed Professor of BioSciences in 2012, and first joined the Rice University faculty in 1998. He received his Ph.D. degree in Molecular Biophysics and Biochemistry from Yale University in 1988.

Dr. Shamoo's research lab studies the dangerous rise of multi-drug resistant bacteria. With multi-drug resistant bacteria becoming increasingly common in hospitals, antibiotic resistance has threatened to return us to a pre-antibiotic era that would completely undermine modern medicine. His work seeks to elucidate the underlying biophysical principles of adaptation within bacterial populations during adaptive evolution. His group uses a combination of experimental evolution, biochemistry, molecular biophysics and genomics to link changes in protein structure and function to their resulting phenotypes within evolving populations. They go on to extend these physicochemical principles to predict the success or failure of specific adaptive alleles undergoing antibiotic selection. The identified evolutionary trajectories to antibiotic resistance serve as rich sources of insight for biomarker discovery as well as understanding the mechanisms of resistance in pathogens.

Samuel Shelburne, MD, PhD
Professor
Infectious Diseases and Genomic Medicine
Challenging Clinical Cases in Antimicrobial Resistance

Dr. Shelburne did his undergraduate work at Princeton University, his medical school training at the University of Texas Medical Branch, and his residency, chief medical residency, and infectious diseases training at Baylor College of Medicine in Houston, TX. He did a research fellowship (T32 and K08) under Dr. Jim Musser at the Methodist Hospital Research Institute in Houston, TX.

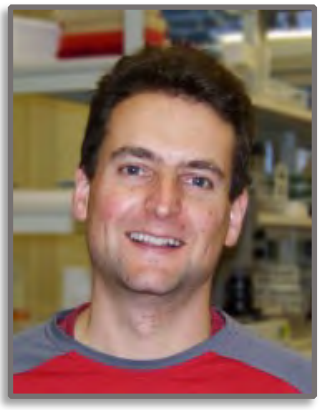
He is the Deputy Chair for Scholarly Activity in the Department of Infectious Diseases at the MD Anderson Cancer Center. His laboratory is interested in factors that determine the incidence and clinical outcomes of bacterial infections. His work mainly focuses on the genomics and signal transduction of group A Streptococcus to cause serious human infections.

The Shelburne laboratory also researches:

- The epidemiology of invasive staphylococcal and group B streptococcal infections in adults
- The impact of the microbiome on infections in immunocompromised patients
- Genetic factors driving antimicrobial resistance amongst bacteria causing human infections

Maria Souli, MD, PMP
Clinical Trials Project Leader II
PHAGE Trial

Dr. Souli obtained her M.D. and Ph.D. from the National and Kapodistrian University of Athens, in Greece. She is specialized in Internal Medicine and Infectious Diseases and was an Associate Professor at the University of Athens until 2019. Dr. Souli is the author or coauthor of more than 90 scientific publications and has written numerous book chapters and scientific abstracts. She has been an invited speaker in more than 120 workshops, seminars, post-graduate courses, symposia, and scientific conferences in national and international meetings and has received several honorary distinctions from Academia and Scientific Societies. Her research interests include the investigation of mechanisms of antibacterial resistance and evaluation of treatment for multi-drug resistant Gram-negative infections. Currently, she is affiliated with Duke University/Duke Clinical Research Institute and works with the Antibacterial Resistance Leadership Group (ARLG) in the management of clinical trials.



Jim Spencer, PhD
Professor
Bacteriology

Carbapenemases in the 21st Century

Jim Spencer is Professor of Bacteriology in the School of Cellular and Molecular Medicine at the University of Bristol. Jim obtained a BSc. Honours degree in Natural Sciences from Cambridge University and a PhD in Biochemistry from the University of Bristol, where he studied protein folding under the supervision of the late Professor Tony Clarke. After postdoctoral research at the National Institute for Medical Research in Mill Hill, London (the forerunner to the Francis Crick Institute) he obtained a Beit Memorial Fellowship for Medical Research to study the action of beta-lactamases, working at the Universities of Bristol and, in the laboratory of Wladek Minor, Virginia. He joined the academic staff in the School of Cellular and Molecular Medicine at the University of Bristol in 2005 and was promoted to Full Professor in January 2021. His current research interests focus on molecular mechanisms of bacterial resistance to antibiotics, particularly beta-lactams and polymixins; while also encompassing development of novel antimicrobials and the interactions of bacteria with nanomaterials.

Pooja Srinivas
Graduate Student
Molecular and Systems Pharmacology

Lost in Translation: Developing Novel Antibiotics Against Bacterial Ribosome Rescue

Pooja is a fifth-year graduate student in the Molecular and Systems Pharmacology at Emory University. Pooja received her bachelor's degree in molecular and cell biology at the University of California, Berkeley, before moving to Atlanta to pursue her PhD in in the lab of Dr. Christine Dunham. In the Dunham lab, Pooja studies various aspects of bacterial translation, focused primarily on the structural basis of ribosome rescue, through single-particle cryo-electron microscopy. Pooja was appointed to the Emory T32 Antimicrobial Resistance and Therapeutic Discovery Training Program in June 2020.

Nicholas Turner, MD, MHSc
Assistant Professor
Division of Infectious Diseases

DOTS Trial: Dalbavancin as an Option for Treatment of Staphylococcus aureus Bacteremia

Dr Turner completed a combined residency in Internal Medicine & Pediatrics, followed by an infectious diseases fellowship at Duke University. As an ARLG research fellow during the last 2 years of his infectious diseases training, he completed a Master's in Clinical Research – modeling the evolving epidemiology of C. difficile across a network of regional hospitals for his thesis. As a true ID generalist, he maintains diverse clinical and research roles – including serving as Associate State TB Controller for North Carolina, as an epidemiologist for the Duke Infection Control Outreach Network, a sub-investigator on a CDC Epicenters grant studying C. difficile prevention measures, and as an ARLG Trialist in Training currently involved in several ongoing clinical trials (including rapid penicillin allergy assessment, dalbavancin for S. aureus bacteremia, and COVID therapeutics).

Paul E. Turner, PhD
Rachel Carson Professor of
Ecology & Evolutionary Biology,
Director of Quantitative Biology Institute and
Program in Physics, Engineering & Biology

Is Phage Therapy Here to Stay?
Keynote Lecture

Paul E. Turner is the Rachel Carson Professor of Ecology and Evolutionary Biology at Yale University. He holds a secondary appointment in the Microbiology Graduate Program at Yale School of Medicine and is an affiliate member of the Microbial Sciences Institute at Yale West Campus. The main focus of Turner's research is to study the evolutionary genetics and genomics of microbes, especially virus ability to adapt (or not) to biotic and abiotic environmental changes at all levels of biological organization: molecules, proteins, cells, populations, communities and ecosystems. This work is highly interdisciplinary, employing molecular biology, microbiology, computational biology, genomics and mathematical-modeling approaches, and especially experimental evolution studies under controlled lab conditions. The Turner lab group studies a wide variety of RNA and DNA viruses, especially bacteriophages that specifically infect bacteria, as well as vector-borne viruses that are transmitted by arthropods such as mosquitoes. His basic research uses rapidly-evolving microbes to test fundamental topics in biology, such as the evolution of genetic exchange (sex); the evolution of specialist versus generalist resource-use; the evolution of thermotolerance; and the evolution of genetic robustness (phenotypic constancy despite mutational change). In addition, his studies concern the ecology and evolution of infectious diseases, such as testing theoretical predictions of pathogen evolvability and emergence on novel hosts. Lastly, Turner studies evolutionary medicine, especially the use of 'evolution-thinking' to better understand human illness and to discover novel disease treatments. These applied projects concern the development of new virus-based therapies to treat antibiotic-resistant bacteria or to attack cancerous tumors. In collaboration with colleagues at Yale New Haven Hospital, Turner has led efforts to secure U.S. FDA approval for using viruses in emergency personalized-treatment of human patients suffering from multi-drug resistant bacterial infections. This work led him to found a startup company based in San Francisco, Felix Biotechnology, which specializes in commercially developing viruses as novel therapeutics, and in clinical trials targeting antibiotic-resistant pathogenic bacteria. The first clinical trial started in early 2021.

Yale University

Turner received his undergraduate Biology degree in 1988 from the University of Rochester and earned his Ph.D. in Zoology (certificate in Microbial Ecology and Evolution) in 1995 from Michigan State University, where Richard E. Lenski served as his thesis advisor. He conducted postdoctoral training via an Intramural Research Training Award Postdoctoral Fellowship with Jeffrey Cohen at National Institutes of Health in Bethesda, MD; a National Science Foundation-NATO Postdoctoral Fellowship with Santiago Elena at the University of Valencia in Spain; and a National Science Foundation Minority Postdoctoral Fellowship with Lin Chao at the University of Maryland-College Park. Turner joined Yale University in 2001 as an Assistant Professor and gained tenure as an Associate Professor in 2007. He then became Full Professor in 2011, before receiving an endowed professorship in 2017. Turner served three years as Director of Graduate Studies and seven years as Departmental Chair of Ecology and Evolutionary Biology at Yale University. In addition, Turner served 1.5 years as Yale's Interim Dean of Science, and served three years as Chair of the Biological Sciences Tenure Promotion Committee, which handles cases for both Yale University and Yale School of Medicine. Currently, Turner is Director of Yale's Quantitative Biology Institute and Program in Physics, Engineering & Biology.

Turner has published over 100 peer-reviewed journal articles, including papers in prestigious scientific journals such as Nature, Science, and Proceedings of the National Academy of Sciences USA. He has also published numerous book chapters, as well as reports for government agencies and private industries. His external awards include competitive grants from private foundations such as Cystic Fibrosis Foundation, the Howard Hughes Medical Institute, Bill & Melinda Gates Foundation, and the Blavatnik Family Foundation, as well as from U.S. federal agencies including National Institutes of Health, National Science Foundation, and NASA. Turner has served as associate editor for scientific journals such as Virus Evolution, Evolution (International Journal of Organic Evolution), and Evolution, Medicine and Public Health. His service to the profession includes Chair of the American Society for Microbiology (ASM) Division on Evolutionary and Genomic Microbiology, as well as membership on the National Science Foundation's Biological Sciences Advisory Committee, ASM Committee on Minority Education, and multiple National Research Council advisory committees. Turner is active in science-communication outreach to the general public, and in programs where faculty collaborate with K-12 teachers to improve STEMM education in underserved public schools.

Turner's honors include a Career Enhancement Fellowship from the Woodrow Wilson Foundation; the E.E. Just Endowed Research Fellowship, and William Townsend Porter Award from Marine Biological Laboratory in Woods Hole, MA; and as a representative in the United States delegation at the joint U.S.A.-Russia Workshop on Infectious Disease held in Novosibirsk, Russia. Turner has delivered numerous invited plenary and memorial lectures at international science conferences, and honorary lectures at colleges and universities. Turner was elected Chair of the Gordon Research Conference on Microbial Population Biology (2013), and Chair of the CNRS Jacques Monod Conference on Viral Emergence (2019). Turner is President-elect of the International Society for Evolution, Medicine and Public Health, and he is an elected Fellow of the National Academy of Sciences, Fellow of the American Academy of Arts & Sciences, and Fellow of the American Academy of Microbiology.

Vijisha C Vijayan, BDS, MPH
Epidemiologist Specialist
LTCF/NH Team

Nursing Homes Response to COVID-19: Lessons Learned

Vijisha C Vijayan currently serves as an Epidemiologist Specialist with Houston Health Department's COVID-19 Response Team. She holds a master's degree in Public Health (MPH) from the UTHealth School of Public Health, with a major in Epidemiology and a bachelor's degree in Dental Surgery (BDS). She possesses over 7 years of experience providing public health services and direct patient care in clinical settings. In her current position with HHD, she assists nursing homes and long-term care (LTC) facilities in their response to COVID-19. She has a proven track record working on prevention and control of COVID-19 infections. Additionally, she is proficient in infectious disease surveillance, outbreak investigation, disease intervention and mitigation.

Nathan Wiederhold, PharmD
Professor
Pathology and Laboratory Medicine

Antifungal Susceptibility Testing of Molds: When, Why, and How

Nathan Wiederhold, PharmD, is a Professor in the Department of Pathology and Laboratory Medicine at UT Health San Antonio. He also serves as the Director of the Fungus Testing Laboratory, an academic reference mycology laboratory for clinical diagnostic testing. Dr. Wiederhold is also highly involved in pre-clinical studies of antifungal agents and assays for the diagnosis of invasive fungal infections, and currently serves as a Co-Principal Investigator on NIH/NIAID contracts that provide in vitro and in vivo pre-clinical resources to the research community for the evaluation of novel antibiotic and antifungal agents. In addition to his clinical and research responsibilities, Dr. Wiederhold serves as an Associate Editor for the Journal of Antimicrobial Chemotherapy and is on the editorial board for Antimicrobial Agents and Chemotherapy. He is also a member of the Clinical and Laboratory Standards Institute (CLSI) Antifungal Susceptibility Subcommittee.

Lynn Zechiedrich, PhD
Kyle and Josephine Morrow Chair in Molecular Virology and Microbiology
Fluoroquinolones, Topoisomerases, and Supercoiled DNA

Lynn Zechiedrich, PhD, is the Josephine and Kyle Morrow Chair and Professor of Molecular Virology and Microbiology and is a Professor in the Department of Biochemistry and Molecular Biology and the Department of Pharmacology and Chemical Biology at Baylor College of Medicine (BCM). Dr. Zechiedrich co-directs the Gulf Coast Consortium on Antibiotic Resistance, the Texas Medical Center Training Program in Antimicrobial Resistance, and the Quantitative and Computational Biosciences inter-institutional graduate program at BCM. She was BCM's BRASS Mentor of the Year in 2013. In 2020, she was the recipient of the Barbara and Corbin J. Robertson, Jr. Presidential Award for Excellence in Education. Dr. Zechiedrich studies DNA topoisomerases and the antibiotics that target them. Because these drugs target the ternary complex of supercoiled DNA-topoisomerase-drug, she invented methods to generate and purify experimentally tractable supercoiled circles of DNA to study how topoisomerases recognize DNA supercoiling, how fluoroquinolones inhibit topoisomerases, and to use in screens for new antibiotics. An elected Fellow in the National Academy of Inventors, Dr. Zechiedrich holds multiple patents that are licensed to the company she founded, Twister Biotech, Inc. She was a Burroughs Wellcome Fund New Investigator and was funded by a Human Frontier Science Program Research Grant. She is currently funded by grants from the National Institutes of Health and the National Science Foundation. Committed to an environment of inclusive excellence, Dr. Zechiedrich was a founding Ambassador for Inclusion and Equity at BCM, receiving a Woman of Excellence award in 2017 for this work. She is the principal flutist in the American Prize-winning Texas Medical Center Orchestra.

Poster Presentation Talks Listed Alphabetically

First Name	Last Name	Institution	Poster Title	Day of presentation
Feroz	Ahmed	UT Arlington	*A Role for Outer Membrane Lipoproteins in Maintaining Cell Envelope Homeostasis in Stress*	1
Eva	Amenta	BCM	*Implementation Outcomes in an Antibiotic Stewardship Program (Kicking CAUTI) in Four Veterans Hospitals Correlated with Clinical Outcomes*	2
Advait	Balaji	RU	*SeqScreen: Accurate and Sensitive Functional Screening of Pathogenic Sequences via*	2
Lina	Carvajal	Universidad El Bosque, Bogotá	*Mercury Resistance and Genetic Determinants in Methicillin-Resistant*	1
Ayan	Chatterjee	UTH	*The Role and Dynamics Of Ethanolamine-Utilizing Bacterial Microcompartments*	2
Justin	Clark	BCM	*Genomic Analysis of Bacteriophage for Therapeutic Use: A Beginner's Guide*	1
Sofia	Costa	Universidade NOVA de Lisboa	*Increased Virulence Potential Among Antimicrobial Resistant Coagulase-Positive Staphylococci Associated with Animal Pyoderma*	2
Kristen	Curry	RU	*Emu: Species-level Microbial Community Profiling for Full-Length Nanopore 16S Reads*	3
Helen	Ding	Parkland Health & Hospital System	*Institutional Prevalence of Drug-Resistant Pathogens in Community-Acquired Pneumonia*	3
Brianna	Eales	UH	*Impact of Metal Limitation on in vitro Growth Rate of A. baumannii*	1

Poster Presentation Talks Listed Alphabetically

First Name	Last Name	Institution	Poster Title	Day of presentation
Sam	Erickson	UTH	*Validation of a Disk Elution Synergy Testing Method for Clinical Labs to Determine*	3
Taryn	Eubank	UH	*An Epidemiologic Exploration of Vancomycin Resistance in Clostridioides difficile*	2
Michael	Fischer and Rachel Singer	Texas Department of State Health Services	*Antibiotic Stewardship Policies in Texas Nursing Homes, 2021*	3
Paula	Gagetti	Instituto Nacional de Enfermedades Infecciosas	*Staphylococcus pseudintermedius's PBP4 is Directly Associated with the Dissociated Oxacillin and Cefoxitin Phenotype*	1
Marc	Gohel	MDA	*Using Whole Genome Sequencing to Genetically Profile and Analyze Escherichia coli Isolates with Varying Resistance to β-lactam/β-*	2
Sonia	Gomez	Laboratorio Nacional de Referencia en Resistencia a los Antimicrobianos	*Carbapenemase-producing Extraintestinal Pathogenic Escherichia coli from Argentina: Clonal Diversity and Predominance of Hyperepidemic Clones CC10 and CC131*	2
Carmen	Gu Liu	BCM	*Lighting The Viral Dark Matter: Revealing Earth's Cryptic Genosphere Through Environmental Sampling*	1
Holly	Hoffman	Paratekpharma	*Comparison of Healthcare Resource Utilization (HRU) Among Adult Patients Treated with Omadacycline (OMC) for*	2

Poster Presentation Talks Listed Alphabetically

First Name	Last Name	Institution	Poster Title	Day of presentation
Kara	Hood	HMRI	*Topology of LiaF from Enterococcus faecalis Suggests Interaction with LiaX*	3
Joseph	Hornak	UTMB	*Delafloxacin in Clinical Practice: A Single Center Study*	2
Tayler	Hunter	UTH	*Investigating the Efficiency of Antibiotic Pocket Irrigants During Breast Reconstruction*	3
Allison	Judge	BCM	*Mapping the Determinants of Antibiotic Catalysis and Substrate Specificity of CTX-M β-lactamases*	3
Alex	Kang	RU	*Mechanism of Carbapenem Resistance in Pseudomonas aeruginosa Cystic Fibrosis Isolates*	1
Iordanis	Kesisoglou	UH	*Seeing the Invisible: Deciphering Spectrophotometry-Based Time-Kill Measurements to Guide the Design of Antibiotic Dosing Regimens*	2
Jordan	Loomis	Parkland Health & Hospital System	*Oral Antibiotic Stepdown Therapy for Uncomplicated Streptococcal Bloodstream*	1
Jacob	McPherson	UH	*A Clostrioides difficile Capillary Electrophoresis-Based PCR Ribotyping Data Analysis Pipeline, Database and Data Visualization Server*	3
Heer	Mehta	RU	*Mutational Switch-Backs Can Accelerate Evolution of Francisella to a Combination of Ciprofloxacin and Doxycycline*	1
Trevor	Moore	UTH	*Temporal Changes in Antibiotic Susceptibility of Group B Streptococcal Isolates from Young Infants with Invasive Infection: 1970-2020*	3

Poster Presentation Talks Listed Alphabetically

First Name	Last Name	Institution	Poster Title	Day of presentation
Analisa	Narro	UTH	*Bacteriophage-Containing Biodegradable Microsphere Technology to Treat Osteomyelitis*	1
Paul	Nicholls	BCM	*Antibiotic Interactions with Bacteriophage in Pseudomonas Biofilms and Planktonic Environments*	2
Mike	Nute* not presenting, Astract and poster included	RU	*A Pan-Genome Analysis of C. difficile Clinical Isolates with Emphasis on Hypervirulent Strain RT027*	3
Melissa	O'Neal	HMRI	*Case Report: Chromosomally Integrated HHV-6B in a Solid Organ Transplant Patient*	1
Diana	Panesso	HMRI	*Role of the LiaF in the LiaR-Mediated Response Against Daptomycin in Multidrug-*	2
Jamie	Peña	MDA	*Characterization of Pathogens Recovered from a Dual Blood Culture System Utilized in Patients with Cancer at a Large Academic Cancer Institution*	3
Jinnethe	Reyes	Universidad El Bosque	*Intestinal Microbiome Composition and Carriage of Multidrug-resistant Organisms among ICU Patients in*	1
SANDRA	Rincón	Universidad El Bosque	*Genomic Characterization and Epidemiology of the Cefazolin Inoculum Effect in Methicillin-*	2
Elizabeth	Sabroske	UTH	*Phenotypic and Genotypic Changes Over Time in Serotype IV GBS Strains*	3
Justin	Schmetterer	Presbyterian Healthcare Services	*NDM-5 Metallo-beta-lactamase in Patient Traveling from India: Superposition Aztreonam and Ceftazidime-Avibactam in vitro Testing and Molecular Sequencing*	1

Poster Presentation Talks Listed Alphabetically

First Name	Last Name	Institution	Poster Title	Day of presentation
Jung	Seo	BCM	*Candida Sternal Wound Infections After Cardiac Operations: Uncommon but Deadly*	2
William	Shropshire	MDA	*Diversity of Carbapenem Resistant Mechanisms Distributed Across Enterobacterales Blood Stream Infections at MD Anderson*	3
Shelby	Simar* not presenting, Astract and poster included	UTH	*Emergence of Tn1549-mediated vanB vancomycin Resistance in Multidrug-resistant Enterococcus faecalis ST6 in the United States*	1
Garima	Singh	UH	*Trick and Treat: Intermittent Antibiotic Dosing to Eradicate Persister Bacteria*	2
Rita	Sobraal	NOVA University Lisbon	*Skf System, a Promising New Bacteriocin System of Staphylococcus aureus*	3
Xinhao	Song	RU	*Developing Methyl Halide Transferase-Based Gas Reporter as a Novel Growth Quantification Approach for Bacteria in Emulsion Droplets*	3
Benjamin	Strope	MDA	*Molecular Characteristics of Emergent Extended-Spectrum Beta-Lactamase Escherichia Coli Infections at MD Anderson Cancer Center*	3
Austen	Terwilliger	BCM	*The TAILΦR Initiative at Year One: Personalized Medicine for Dynamic Infections*	2
Truc	Tran	HMRI	*LiaX is Essential for Cell Envelope Adaptation via the LiaFSR System in Enterococcus faecium*	3
Luis	Vega	UTH	*The Integrative Conjugative Element ICESpyM92 Contributes to Pathogenicity of*	2

Poster Presentation Talks Listed Alphabetically

First Name	Last Name	Institution	Poster Title	Day of presentation
M Hassan	Virk	HMRI	*Dynamics Of Colonization and Infection By Multidrug-Resistant Pathogens in Immunocompromised and Critically Ill Patients (DYNAMITE): Preliminary Results*	3
M Hassan	Virk and Rachel Atterstorm	HMRI	*Customizing Populus Plus Lab Inventory Management System for Large Multi Center Observational trial: DYNAMITE*	3
Jennifer	Walker	UTH	*Characterization of the Antimicrobial Susceptibility Patterns and Biofilm Formation During Staphylococcal Medical*	1
Yizhe	Zhang	RU	*Directed Evolution of Wild Streptomyces towards Antimicrobial Production through Co-Culture with Competitor Pathogens in Microfluidic Droplets*	3
Yue	Zhou	RU	*Structural and Biochemical Studies of MurAA, an Enolpyruvate Transferase that Contributes to*	1
Jacob	Zulk	BCM	*Phage Resistance Accompanies Reduced Fitness of Uropathogenic E. Coli in the Urinary Environment*	2

Poster Presentation Talks Day 1

First Name	Last Name	Institution	Poster Title	Day of presentation
Feroz	Ahmed	UT Arlington	*A Role for Outer Membrane Lipoproteins in Maintaining Cell Envelope Homeostasis in Stress*	1
Lina	Carvajal	Universidad El Bosque, Bogotá	*Mercury Resistance and Genetic Determinants in Methicillin-Resistant Staphylococcus aureus USA300-Latin-American Variant Recovered from Latin-American Hospitals*	1
Justin	Clark	BCM	*Genomic Analysis of Bacteriophage for Therapeutic Use: A Beginner's Guide*	1
Brianna	Eales	UH	*Impact of Metal Limitation on in vitro Growth Rate of A. baumannii*	1
Paula	Gagetti	Instituto Nacional de Enfermedades Infecciosas	*Staphylococcus pseudintermedius's PBP4 is Directly Associated with the Dissociated Oxacillin and Cefoxitin Phenotype*	1
Carmen	Gu Liu	BCM	*Lighting The Viral Dark Matter: Revealing Earth's Cryptic Genosphere Through Environmental Sampling*	1
Alex	Kang	RU	*Mechanism of Carbapenem Resistance in Pseudomonas aeruginosa Cystic Fibrosis Isolates*	1
Jordan	Loomis	Parkland Health & Hospital System	*Oral Antibiotic Stepdown Therapy for Uncomplicated Streptococcal Bloodstream Infections*	1
Heer	Mehta	RU	*Mutational Switch-Backs Can Accelerate Evolution of Francisella to a Combination of Ciprofloxacin and Doxycycline*	1
Analisa	Narro	UTH	*Bacteriophage-Containing Biodegradable Microsphere Technology to Treat Osteomyelitis*	1

Poster Presentation Talks Day 1

First Name	Last Name	Institution	Poster Title	Day of presentation
Melissa	O'Neal	HMRI	*Case Report: Chromosomally Integrated HHV-6B in a Solid Organ Transplant Patient*	1
Jinnethe	Reyes	Universidad El Bosque	*Intestinal Microbiome Composition and Carriage of Multidrug-resistant Organisms among ICU Patients in Colombian hospitals*	1
Justin	Schmetterer	Presbyterian Healthcare Services	*NDM-5 Metallo-beta-lactamase in Patient Traveling from India: Superposition Aztreonam and Ceftazidime-Avibactam in vitro Testing and Molecular Sequencing*	1
Shelby	Simar* not presenting, Astract and poster included	UTH	*Emergence of Tn1549-mediated vanB vancomycin Resistance in Multidrug-resistant Enterococcus faecalis ST6 in the United States*	1
Jennifer	Walker	UTH	*Characterization of the Antimicrobial Susceptibility Patterns and Biofilm Formation During Staphylococcal Medical Device Infection*	1
Yue	Zhou	RU	*Structural and Biochemical Studies of MurAA, an Enolpyruvate Transferase that Contributes to Cellular Fitness During Daptomycin Attack in Enterococcus faecium*	1

A Role for Outer Membrane Lipoproteins in Maintaining Cell Envelope Homeostasis in Stress

Ahmed F[1], Gray J[2], Vollmer W[2], Boll JM[1]
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Background: The Gram-negative outer membrane is an asymmetric lipid bilayer that acts as a barrier to limit antibiotic entry. The inner leaflet consists of glycerophospholipids, while the outer monolayer is enriched in lipopolysaccharide (LPS) or lipooligosaccharide (LOS). The LPS/LOS moiety was canonically thought to be essential for Gram-negative survival and has been targeted by antimicrobial therapeutics. However, the critical threat nosocomial pathogen, *Acinetobacter baumannii* can shutdown LOS biosynthesis (LOS^-) to gain resistance to the last resort antibiotic colistin, and other clinically important antimicrobials.

Hypothesis/goals: Here, we test the hypothesis that specific lipoproteins are essential for survival of colistin resistant LOS^- *A. baumannii*, and that they also contribute to outer membrane homeostasis in stress. We also aimed to define specific lipoproteins that attach the outer membrane to the peptidoglycan cell envelope layers, which increases the mechanical load-bearing potential of the cell envelope.

Methods: Peptidoglycan was purified from *A. baumannii* cells and attached proteins were analyzed using mass spectrometry to determine the peptidoglycan associated proteome. Genetic mutants were constructed in candidate lipoprotein genes and LOS^- survival assays were performed to determine the requirement for each lipoprotein. Permeability and outer membrane vesiculation assays were performed to calculate the impact of each lipoprotein on outer membrane integrity maintenance.

Results: Proteomic analysis of peptidoglycan attached proteins showed two putative outer membrane lipoproteins (denoted as Lpp1 & Lpp2) were covalently attached. Notably, both Lpp1 & Lpp2 encode signature C-terminal lysine residues, a motif essential for LD-transpeptidase-dependent covalent attachment of the lipoprotein to the meso-diaminopimelic acid (meso-DAP) residue in peptidoglycan stem peptides. Covalent tethering of the outer membrane to the peptidoglycan via lipoproteins likely stabilizes the cell envelope when LOS is not produced. Mutational analysis of Lpp1 and Lpp2 increased outer membrane vesicle production relative to wild type, which supports a model where lipoprotein attachment stabilizes the outer membrane. We have found that Lpp1 is expressed in growth and stationary phase, while Lpp2 is only expressed in stasis, suggesting separate roles for the lipoproteins in outer membrane assembly.

Conclusions: Together, our studies show that *A. baumannii* encodes two lipoproteins that physically link the outer membrane to the cell wall to increase cell envelope stability in response to outer membrane defects.

Acknowledgements This work is supported by the National Institute of Health (R35 GM143053).

A Role for Outer Membrane Lipoproteins in Maintaining Cell Envelope Homeostasis in Stress

Feroz Ahmed[1], Joe Gray,[2] Waldemar Vollmer[2], Joseph M. Boll[1]

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2. . Centre for Bacterial Cell Biology, Biosciences Institute, Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction

The lipidA moiety of lipopolysaccharide/lipooligosaccharide (LPS/LOS) was canonically thought to be essential for Gram-negative survival and has been targeted by antimicrobial includes polymyxin E (colistin). However, the critical threat nosocomial pathogen, *Acinetobacter baumannii* can shutdown LOS biosynthesis (LOS$^-$) to gain resistance to the last resort antibiotic colistin, and other clinically important antimicrobials. Here, we test the hypothesis that specific lipoproteins are essential for survival of colistin resistant LOS$^-$ *A. baumannii*, by maintaining outer membrane homeostasis in stress. Proteomic analysis of peptidoglycan attached proteins showed two putative outer membrane lipoproteins (denoted as Lpp1 & Lpp2) were covalently attached to peptidoglycan. Both Lpp1 & Lpp2 encode signature C-terminal lysine residues, a substrate for LD-transpeptidase-dependent covalent attachment of the lipoprotein to the meso-diaminopimelic acid (meso-DAP) residue in peptidoglycan stem peptides. Covalent tethering of the outer membrane to the peptidoglycan via lipoproteins likely stabilizes the cell envelope when LOS is not produced. Mutational analysis of Δ*lpp1* and Δ*lpp2* increased outer membrane vesicle production and increased permeability relative to wild type, which supports a model where lipoprotein attachment stabilizes the outer membrane. We have found that Lpp1 is expressed in growth and stationary phase, while Lpp2 is only expressed in stasis, suggesting separate roles in outer membrane assembly.

Lipoproteins re-decorate the cell envelope in stress

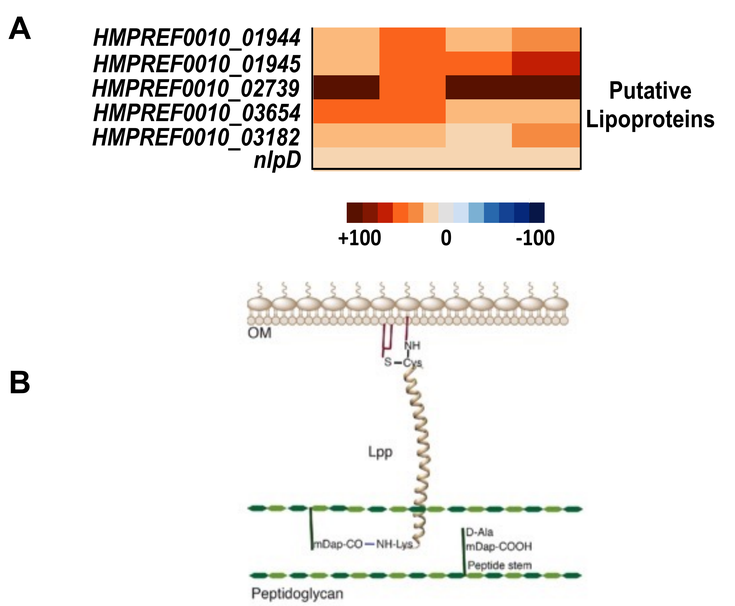

Fig 1: A) Heat map illustrating the altered expression of putative lipoproteins in multiple *A. baumannii* strains. B) The lipoprotein Lpp is anchored to he outer membrane via a lipid moiety also bound to meso-DAP residues in the peptide stems of the peptidoglycan via its C-terminal lysine residue.

The peptidoglycan-attached proteome of *A. baumannii*

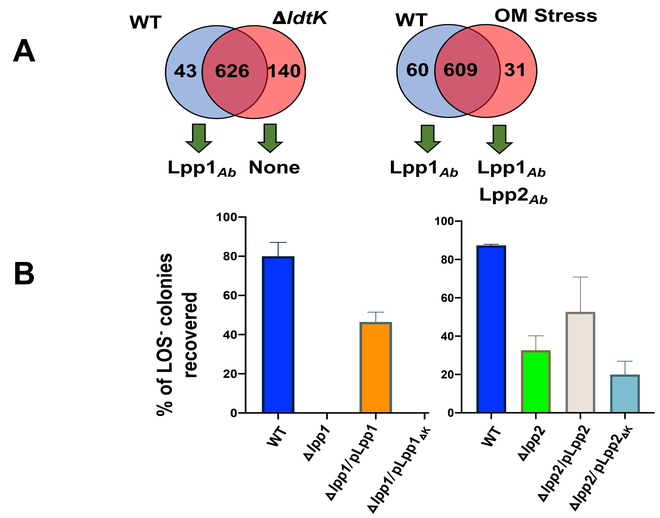

Fig 2: A) Peptidoglycan associated proteins of WT vs Δ*ldtK* and WT vs stress were determined using mass spectrometry B) Percentage of recovered LOS$^-$ *A. baumannii* after colistin selection on WT vs Δ*lpp* mutants and complemented strains.

Biochemical validation of LD-transpeptidase-dependent attachment of lipoproteins to peptidoglycan

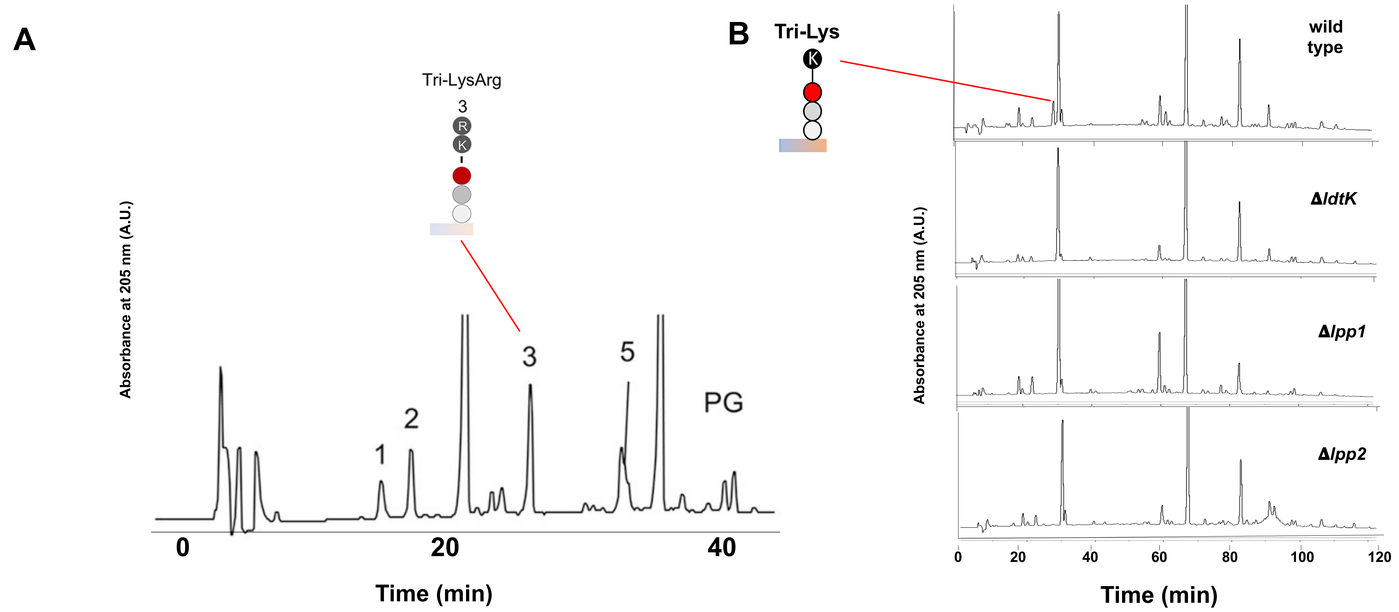

Fig 3: A) Chromatogram highlighting the Arginine-Lysine peak in muropeptide composition from isolated peptidoglycan of *E.coli*. B) Chromatogram highlighting the Lysine peak in muropeptide composition from isolated peptidoglycan of wild type *A. baumannii* and mutants (Δ*ldtk*, Δ*lpp1* and Δ*lpp2*).

Lipoproteins contribute outer membrane stability

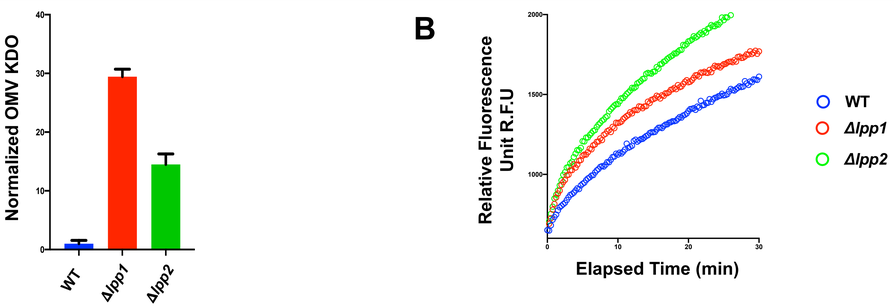

Fig 4: A) Relative quantification of outer membrane vesicles (OMVs) Kdo concentrations in Δ*lpp1* and Δ*lpp2* relative to WT. B) Permeability assays using ethidium bromide (EtBr) of Δ*lpp1* and Δ*lpp2* relative to WT over 30 mins.

Conclusion

Proteomic analysis of peptidoglycan attached proteins showed two putative lipoproteins were attached to peptidoglycan in wild type but not Δ*ldtk*. Notably, both lipoproteins encode C-terminal lysine residues, which are attachment sites for LD-transpeptidase-dependent covalent attachment of lipoproteins to meso-DAP in peptidoglycan stem peptides. Physically tethering the outer membrane to peptidoglycan via lipoproteins increase cell envelope stability in response to outer membrane defects.

References

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5. Geisinger, E. et al. A global regulatory system links virulence and antibiotic resistance to envelope homeostasis in *Acinetobacter baumannii*. PLOS Pathogens. 14(5), E1007030 (2018)

Acknowledgements: This work is supported by the National Institute of Health (R35 GM143053)

Mercury Resistance and Genetic Determinants in Methicillin-Resistant Staphylococcus aureus USA300-Latin-American Variant Recovered from Latin-American Hospitals

Carvajal LP [1], Rincon S[1], Castro BE[1], Ríos R[1], Arias CA[2,3], Diaz L[1], Reyes J[1]

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Background:

MRSA-USA300-LV is a successful clone established in the northern region of South-America, that harbors mercury resistance genes (*mer*) within the COMER-genetic element. Mercury is a highly toxic heavy-metal with no beneficial biological activity. The *mer* gene cluster contribute with detoxification of mercurial compounds. Of note, mercury pollution due to mining activities represents a public health and environmental problem in Colombia. In this context, the significance of the presence of *mer* genes in USA300-LV clone is intriguing and we aimed to investigate the genetic elements involved in mercury resistance and their functionality in a collection of MRSA-USA300-LV clinical isolates recovered from three countries in Latin-America.

Materials /Methods:

We study sixty-five bloodstream MRSA-USA300-LV isolates recovered from hospitals in Colombia, Ecuador and Venezuela between 2010-2014. Mercury resistance genes (*merA/merB*) were assessed by PCR. Functionality of *mer* genes was evaluated through Minimum Inhibitory Concentration (MIC) determinations for $HgCl_2$ in TSB at 33°C. Whole-genome sequencing was performed to characterize genetic determinants involved in mercury resistance and their genomic context and elements involved in mobilization of *mer* genes.

Results

merA/merB were identified in 82% of MRSA- USA300-LV isolates (53 out of 65). Moreover, 81% (26 out of 32) and 95% (19 out of 20) of MRSA-USA300-LV from Colombia and Ecuador harbored *merA/merB,* respectively, while, it was identified 65% (8 out of 13) in Venezuelan isolates. All isolates belonged to the CC8 (ST8 and ST923 were found in 98.5% and 1.5%, respectively, of isolates). The presence of *mer* genes were strongly associated to mercury resistance, since all 53 isolates harboring *merA/merB* exhibited $HgCl_2$ MIC of ≥128 μg/mL. Conversely, MRSA lacking *merA/merB* consistently showed MICs of ≤64 μg/mL. Genomic analysis revealed that the *mer* gene cluster was located on the chromosome and associated to SCC*mec* IVc/E and COMER genetic element in all resistant isolates (n=53). Further, among 12 *mer-negative* isolates, 6 harbor the other genes encoded by COMER-element. COMER was absent in the remaining 6 MRSA. Genome context comparison of *mer* positive and negative strains, revealed that IS431 is likely to mediate the acquisition of *mer* genes.

Conclusions

mer genes among USA300-LV were exclusively found in mercury-resistant isolates, supporting their role in mercury resistance. Their chromosomal location suggest co-selection with antibiotic resistance genes. Thus, the *mer* genes might favor dissemination of the USA300-LV clone, in certain regions in Latin America.

Acknowledgements: this study was funded by Universidad El Bosque

Mercury Resistance and Genetic Determinants in Methicillin-Resistant *Staphylococcus aureus* USA300-Latin-American Variant recovered from Latin-American hospitals.

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Abstract

Background: MRSA-USA300-LV is a successful clone established in the northern region of South-America, that harbors mercury resistance genes (*mer*) within the COMER-genetic element. Mercury is a highly toxic heavy-metal with no beneficial biological activity. The *mer* gene cluster contribute with detoxification of mercurial compounds. Of note, mercury pollution due to mining activities represents a public health and environmental problem in Colombia. In this context, the significance of the presence of *mer* genes in USA300-LV clone is intriguing and we aimed to investigate the genetic elements involved in mercury resistance and their functionality in a collection of MRSA-USA300-LV clinical isolates recovered from three countries in Latin-America.

Materials /Methods: We study sixty-five bloodstream MRSA-USA300-LV isolates recovered from hospitals in Colombia, Ecuador and Venezuela between 2010-2014. Mercury resistance genes (*merA/merB*) were assessed by PCR. Functionality of *mer* genes was evaluated through Minimum Inhibitory Concentration (MIC) determinations for $HgCl_2$ in TSB at 33°C. Whole-genome sequencing was performed to characterize genetic determinants involved in mercury resistance and their genomic context and elements involved in mobilization of *mer* genes..

Results: *merA/merB* were identified in 82% of MRSA- USA300-LV isolates (53 out of 65). Moreover, 81% (26 out of 32) and 95% (19 out of 20) of MRSA-USA300-LV from Colombia and Ecuador harbored *merA/merB,* respectively, while, it was identified 65% (8 out of 13) in Venezuelan isolates. All isolates belonged to the CC8 (ST8 and ST923 were found in 98.5% and 1.5%, respectively, of isolates). The presence of *mer* genes were strongly associated to mercury resistance, since all 53 isolates harboring *merA/merB* exhibited $HgCl_2$ MIC of ≥128 µg/mL. Conversely, MRSA lacking *merA/merB* consistently showed MICs of ≤64 µg/mL. Genomic analysis revealed that the *mer* gene cluster was located on the chromosome and associated to SCC*mec* IVc/E and COMER genetic element in all resistant isolates (n=53). Further, among 12 *mer*-negative isolates, 6 harbor the other genes encoded by COMER-element. COMER was absent in the remaining 6 MRSA. Genome context comparison of *mer* positive and negative strains, revealed that IS431 is likely to mediate the acquisition of *mer* genes.

Conclusions: *mer* genes among USA300-LV were exclusively found in mercury-resistant isolates, supporting their role in mercury resistance. Their chromosomal location suggest co-selection with antibiotic resistance genes. Thus, the *mer* genes might favor dissemination of the USA300-LV clone, in certain regions in Latin America.

Background

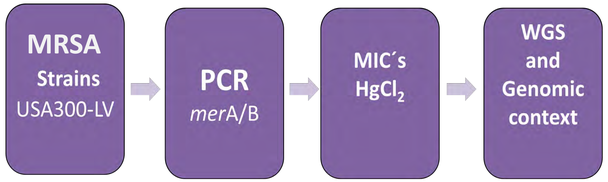
Methicillin resistant *Staphylococcus aureus* USA300-Latin American Variant (MRSA-USA300-LV) is a successful clone established in the northern region of South-America, that harbors mercury resistance genes (*mer*) within the COMER-genetic element$_1$. Mercury is a highly toxic heavy-metal with no beneficial biological activity. The *mer* gene cluster contribute with detoxification of mercurial compounds$_2$. Of note, mercury pollution due to mining activities represents a public health and environmental problem in Colombia$_3$. We previously found a high prevalence of Heavy Metal Resistance (HMR) 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 country[4]

In this context, the significance of the presence of *mer* genes in USA300-LV clone is intriguing and we aimed to investigate the genetic elements involved in mercury resistance and their functionality in a collection of MRSA-USA300-LV clinical isolates recovered from three countries in Latin-America

Aim

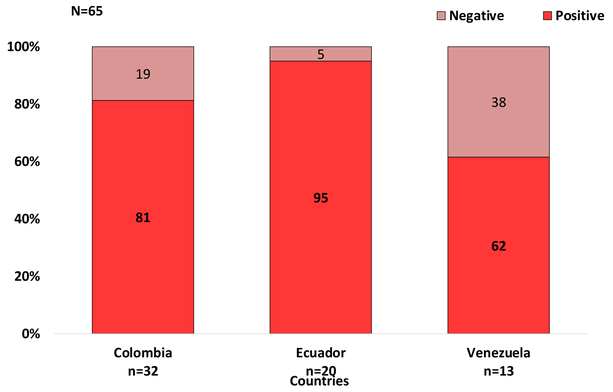
To investigate the genetic elements involved in mercury resistance and their functionality in a collection of MRSA-USA300-LV clinical isolates recovered from three countries in Latin-America.

Methods



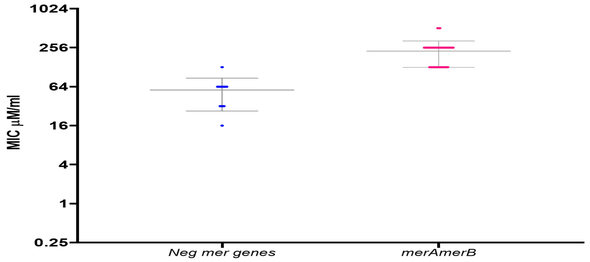
Results

1. *mer*A and *merB* identified by PCR in MRSA USA300-LV isolates



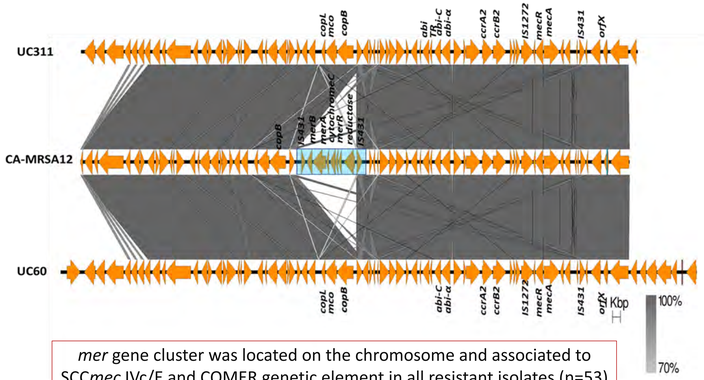
All isolates belonged to the CC8
ST8 and ST923 were found in 98.5% and 1.5%, respectively, of isolates

2. $HgCl_2$ MICs and *mer*A /*mer*B in MRSA USA300-LV isolates



53 isolates harboring *merA/merB* exhibited $HgCl_2$ MIC of ≥128 µg/mL
MRSA lacking *merA/B* consistently showed MICs of ≤64 µg/mL.

3. Genomic Analysis in Representative USA300-LV strains



mer gene cluster was located on the chromosome and associated to SCC*mec* IVc/E and COMER genetic element in all resistant isolates (n=53)

Among 12 *mer*-negative isolates:

- 6 harbor the other genes encoded by COMER-element.
- COMER was absent in the remaining 6 MRSA.
- Genome context comparison of *mer* positive and negative strains, revealed that IS431 is likely to mediate the acquisition of *mer* genes.

Conclusions

- *mer* genes among USA300-LV were exclusively found in mercury-resistant isolates, supporting their role in mercury resistance.
- Their chromosomal location suggest co-selection with antibiotic resistance genes.
- Thus, *mer* genes might favor dissemination of the USA300-LV clone, in certain regions in Latin America.

Funding

Universidad El Bosque

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1. Planet PJ, Diaz L, Kolokotronis SO, et al. Parallel Epidemics of Community-Associated Methicillin-Resistant *Staphylococcus aureus* USA300 Infection in North and South America. J Infect Dis. 2015;212(12):1874–1882.
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3. Palacios-Torres Caballero-Gallardo K, Olivero-Verbel J. Mercury pollution by gold mining in a global biodiversity hotspot, the Choco biogeographic region, Colombia. Chemosphere. 2018;193:421–430.
4. Diaz L, Solano J, Rios R, et al. 1214. High Frequency of genes encoding resistance to Heavy Metals in Methicillin-Resistant *Staphylococcus aureus* (MRSA) endemic lineages from South America. Open Forum Infect Dis. 2018;5(Suppl 1):S368.

Genomic Analysis of Bacteriophage for Therapeutic Use: A Beginner's Guide

Clark JR, Terwilliger AW, Green SI, Salazar KC, Maresso AM

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Background:

The number of deaths that result from antibiotic resistance is rising so rapidly that some predict it will surpass the deaths that result from cancer in less than 30 years. One of the major problems with combating this growing threat is the fact that bacteria can acquire resistance to new antibiotics at a rate orders of magnitude higher than humans develop them. Simply put, new, non-antibiotic, strategies are needed. By far the most advanced and promising alternative to antibiotics is bacteriophage—or phage—therapy. Phage have been used as a therapeutic for nearly a century, even in the US. However, the low cost and availability of antibiotic sapped interest for this therapy. The rise of antibiotic resistance has reversed this, especially in dire cases when antibiotic have failed completely. Against this grim backdrop, we founded of Tailored Antimicrobials and Innovative Laboratories for Phage (Φ) Research (TAILΦR) at Baylor College of Medicine, a phage service center dedicated to advancing the understanding of phage biology and produce therapeutic-grade phage cocktails. An important part of realizing our mission is the analysis of phage genomes. For a variety of reasons, therapeutic phages are expected to be devoid of any virulence factors, antibiotic resistance genes, or a lysogenic life cycle. One of the most efficient and comprehensive ways to screen phages for these undesirable genes is by using Next-Generation sequencing (NGS) and a standard framework of bioinformatic analysis is starting to emerge. Already what is generally considered a minimum set of analyses has emerged and will be explored here.

Goals:

Here we will describe an overview of bioinformatic tools that are typically used to analyze phage for therapeutic use. We will also describe exploratory work that have the potential to be applied in uncovering problematic genes/trains in the future.

Methods:

Methods covered will include assembly and analysis of the phage genomes using short-read sequencing and screening of these assemblies using sequence alignment tools such as *blast* and *hmmer* to search for problematic genes that are unsuitable for use in patient.

Conclusions:

The framework presented here lays out a basic modular overview of how one can analyze a phage for therapeutic use. This framework can also be used when characterizing a phage genome to report on its potential as a therapeutic.

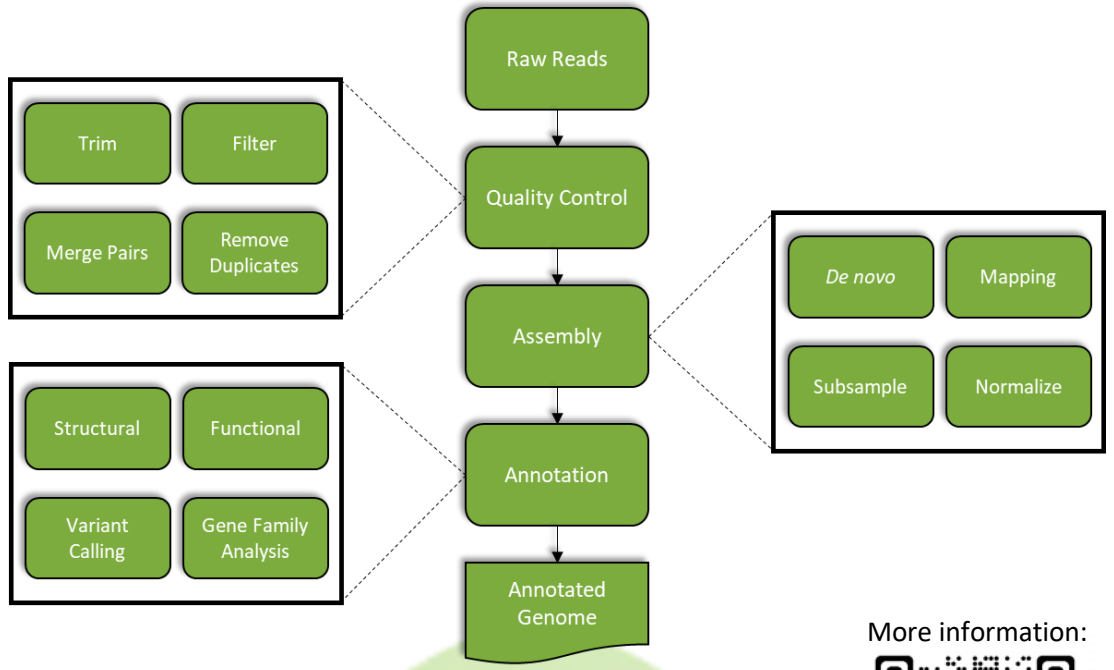
Acknowledgements:

This work is supported by the National Institute of Health grants U19-AI157981, VA I01-RX002595, the Kleberg Foundation and Baylor College of Medicine seed funds awarded to Anthony Maresso and TAILΦR

Genomic Analysis of Therapeutic Bacteriophages

Justin Clark, Austen Terwilliger, Sabrina Green, Keiko Salazar, and Anthony Maresso
TAILΦR Labs, Department of Molecular Virology and Microbiology, Baylor College of Medicine

Background: The number of deaths that result from antibiotic resistance is rising so rapidly that some predict it will surpass the deaths that result from cancer in less than 30 years. One of the major problems with combating this growing threat is the fact that bacteria can acquire resistance to new antibiotics at a rate orders of magnitude higher than humans develop them. Simply put, new, non-antibiotic, strategies are needed. By far the most advanced and promising alternative to antibiotics is bacteriophage—or phage—therapy. Phage have been used as a therapeutic for nearly a century, even in the US. However, the low cost and availability of antibiotic sapped interest for this therapy. The rise of antibiotic resistance has reversed this, especially in dire cases when antibiotic have failed completely. Against this grim backdrop, we founded of Tailored Antimicrobials and Innovative Laboratories for Phage (Φ) Research (TAILΦR) at Baylor College of Medicine, a phage service center dedicated to advancing the understanding of phage biology and produce therapeutic-grade phage cocktails. An important part of realizing our mission is the analysis of phage genomes. For a variety of reasons, therapeutic phages are expected to be devoid of any virulence factors, antibiotic resistance genes, or a lysogenic life cycle. One of the most efficient and comprehensive ways to screen phages for these undesirable genes is by using Next-Generation sequencing (NGS) and a standard framework of bioinformatic analysis is starting to emerge.



Primary Goals:

- Determine lifestyle (lytic vs lysogenic)
  - PHACTs, phage.ai, integrases
- Screen assemblies and annotations for the presence of:
  - Virulence Factors (VFDB, VICTORS, PATRIC VF, etc)
  - Antibiotic Resistance Genes (CARD)
  - Mobile Elements

Secondary Goals:

- Determine phylogeny
- Screen for bacterial contamination
- Verify presence of a single phage
- Verify lack of insertions (variant analysis)

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Impact of Metal Limitation on in vitro Growth Rate of A. baumannii

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Background: Antimicrobial resistance (AMR) is an ever-present problem due to the evolving mechanisms that bacteria use to evade antibiotics. *Acinetobacter baumannii* is one of these antibiotic-resistant pathogens and has become multidrug resistant in recent years. Currently, there is no consensus on the optimal treatment for multidrug-resistant *A. baumannii* infections. Nutrient metals (e.g., iron) are essential to the metabolic functions of *A. baumannii*, and these essential nutrients must be acquired from their host. If the bioavailability of essential nutrients is restricted, this can cause a bacterial stress response and alter their ability to multiply.

Hypothesis/Goals: We expect to demonstrate that metal limitation will result in a compromise to essential bacterial functions.

Methods: To examine bacterial response under metal limiting conditions, bacterial densities of *A. baumannii* ATCC BAA747 are measured longitudinally over 24-hours to determine the growth rate of the bacteria. Variable amounts of an iron chelating agent are added to the samples to create a concentration gradient; a sample without iron chelator is used as a no-treatment control. Bacterial density is measured by an automated process (BacterioScan 216Dx), in which optical signals captured were converted to log CFU/mL by a validated algorithm integrating both absorbance and forward light scattering.

Results: Increasing concentrations of the iron chelating agent showed a trend in impeded bacterial growth compared to the no-treatment group.

Conclusions: Metal micro-nutrient limitation has the potential to be a novel approach for treating high-risk infections due to drug-resistant *A. baumannii.*

Acknowledgements: This research was funded by the National Institutes of Health, grant number R01AI140287-04.

Impact of Metal Limitation on *in vitro* Growth Rate of *A. baumannii*

Brianna M Eales[1], Bing Bai[2], Vincent H Tam[1,2,3]

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[2]Department of Infectious Diseases and Shenzhen Key Laboratory for Endogenous Infections, The 6th Affiliated Hospital of Shenzhen University Health Center
[3]Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy

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Background

- Antimicrobial Resistance (AMR) is emerging as a health care crisis that requires solutions now
- *Acinetobacter baumannii* is a pathogenic bacterium that needs immediate concentrated dedication
- Innovative treatment strategies are needed
- Target an essential nutrient → iron

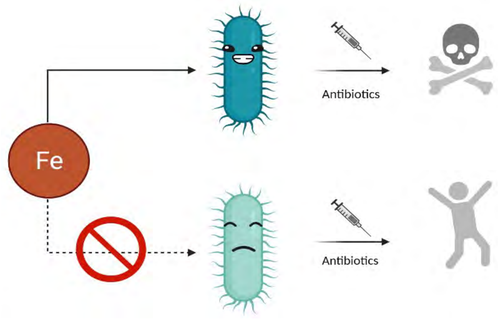

Figure 1. Iron (Fe) is a metal micro-nutrient essential to many metabolic functions. Multidrug-resistant bacteria with access to metals result in fatal infections even with antibiotic interventions (top path). Under metal-limited conditions, bacteria would be rendered more susceptible to antibiotics and antibiotic interventions could result in lower mortality (bottom path).

Hypothesis / Goals

- Demonstrate that metal limitation will result in a compromise to essential bacterial functions.

Methods

BacterioScan 216Dx

- Machine that measures bacterial density of up to 36 samples simultaneously up to 20 hours
- Optical signals are captured every 15 minutes
- Proprietary calculation to convert optical signals to Log CFU/mL
- Samples are kept at 37°C
- Starting bacterial concentration of approximately 5.5 x 10^5 CFU/mL
- Initial inocula verified by quantitative culture

Results

- Increased concentration of iron chelator, DIP, caused an increase of growth hinderance of *A. baumannii*
- Representative graphs are shown for select isolates in Figure 2
- All isolates showed a growth profile difference when comparing the control group 0uM (blue) and 100uM (orange) DIP
- Only one isolate had apparent growth in the 160uM DIP group before 400 minutes

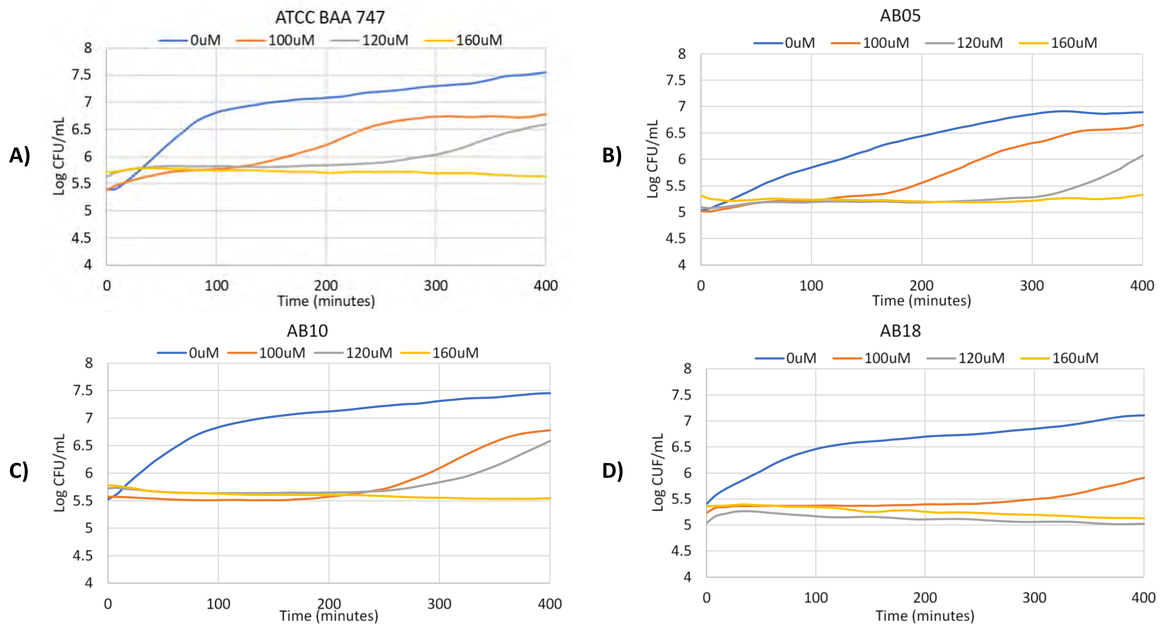

Figure 2. Graphic representation of bacterial growth in Log CFU/mL over 400 minutes in the presence of 0uM (blue), 100uM (orange), 120uM (gray), and 160uM (yellow) DIP. A) laboratory strain ATCC BAA 747; B) Clinical isolate # 05; C) Clinical isolate # 10; D) Clinical isolate # 18.

Materials

2,2' Dipyridyl – DIP

- Iron chelating compound
- Can bind to both Fe^{+2} and Fe^{+3}
- Graphical representation of binding formation shown in Figure 3

Tryptic Soy Broth

- limited iron content
- made with molecular grade water to prevent addition of minerals from the water

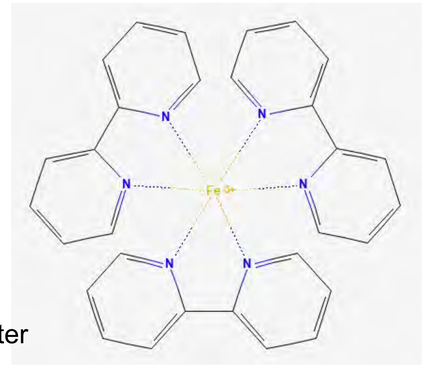

Figure 3. Structure of chelated Fe^{+3} and 3 DIP molecules.

Bacterial Isolates

Control – Laboratory Strain: ATCC BAA 747

- Not Typed
- Not MDR

Isolate #	Gender	Age	Source	MLST[1]	MDR[2]
01	M	39	Respiratory	457	Yes
05	M	80	Respiratory	NT	Yes
10	M	81	Urinary	447	Yes
14	M	50	Abdominal	195	No
18	M	76	Biliary	373	No
19	M	76	Line	NT	No
23	M	74	Respiratory	NT	No
28	M	80	Respiratory	NT	Yes

[1] MLST – Multilocus sequence typing via Oxford Scheme; NT – non-typable
[2] MDR – Multi-drug resistance multidrug resistance, as nonsusceptibility to ≥1 agent in ≥3 antimicrobial categories

Table 1. Clinical isolate characteristics. All isolates were collected from the bloodstream from unique patients between 2011 and 2018. Isolate characterization and antimicrobial susceptibility testing was performed using VITEK®2 system and validated by MALDI-TOF mass spectroscopy.

Discussion

- Response to an iron chelator was shown in a laboratory strain and clinical isolates with both MDR and non-MDR phenotypes
- Evaluate FDA approved chelators to determine if bacterial response is the same as it is in the presence of DIP
- Investigation in response to antibiotics combined with chelation is still required

Conclusion

- Using an iron chelator, there is a relationship between growth hindrance and escalating iron chelator concentration
- Iron limitation has the potential to be a novel approach for treating high-risk infections due to drug-resistant *A. baumannii*

Acknowledgements

This research was funded by the National Institutes of Health, grant number R01AI140287-04.

Staphylococcus pseudintermedius's PBP4 is Directly Associated with the Dissociated Oxacillin and Cefoxitin Phenotype

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Background

Staphylococcus pseudintermedius is an important pathogen responsible for infections in dogs and in humans. The emergence and dissemination of methicillin-resistant *S. pseudintermedius* (MRSP) and their multidrug resistance make difficult the treatment of these pathogens. Cefoxitin (FOX), a β-lactam antibiotic used as a surrogate marker of the *mecA* gene in *S. aureus* and other staphylococcal species, is a strong inducer of the *mecA* operon that is currently utilized to detect methicillin resistance. However, FOX it is not an accurate method of screening for methicillin resistance in *S. pseudintermedius*. Given this limitation, in 2016 the Clinical and Laboratory Standard Institute (CLSI) and recently the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommended the use of the oxacillin (OXA) disk instead of FOX for the detection of methicillin resistance in *S. pseudintermedius.* However, the mechanistic bases linked to FOX failure in detecting methicillin resistance in MRSP is still unknown.

Goals

The purpose of this study was to elucidate the molecular bases of the dissociated phenotype between oxacillin and cefoxitin antibiotics in MRSP.

Methods

A methicillin-susceptible (MSSP8316) and two resistant representative *S. pseudintermedius* strains (MRSP8148, MRSP8150) isolated from canine clinical samples in Argentina were studied. These strains were phenotypically characterized and whole-genome sequenced. The membrane abundance, activity of PBPs and competition assays between β-lactams and bocillin were performed. PBP sequences of MRSP isolates were analyzed and compared with those corresponding to reference strains of *S. aureus* and *S. pseudintermedius*.

RNA-Seq was performed in MRSP8150 cells grown in the absence and presence of subinhibitory concentrations of cephalexin (CEF). Genome-wide transcript sequencing libraries were prepared according to the manufacturer's recommendations (Illumina, San Diego, CA). Differential gene expression was determined using Lasergene (v14) software (DNAStar, Madison, WI), and differences of >3-fold after applying Bonferroni correction were statistically significant.

Results

All four native PBPs (1–4) were distinctly identified in membranes' samples from both MSSP and MRSP strains with the presence in the latter of PBP2A, similarly as in MRSA strains. Competition assays showed that cephalexin bounds PBP2 with high affinity, but no changes were seen in PPB1, PBP3, or PBP4. Increased binding of cefoxitin to PBP4 at different concentrations was observed while no changes were observed for cephalexin and oxacillin. Moreover, we found that in MRSP, PBP1 is the largest with bifunctional transglycosylase and transpeptidase domains, homologous to *S. aureus* PBP2. PBP2 and PBP3 with transpeptidase function and PBP4 with transpeptidase /carboxypeptidase activity. The observed inhibition of PBP4 by FOX in the competition assays strongly suggests that PBP4 is directly related to the dissociated phenotype between OXA and FOX in MRSP.

CEF is an antibiotic extensively used to treat MRSP dog infections; expression analyses conducted in MRSP revealed a decreased expression of *pbp4*, *fmtB*, and *aux2* genes related to peptidoglycan synthesis and cell membrane (*mprF*) in addition to several genes involved in DNA metabolism and strict stress response.

Conclusions

Collectively, this study led to novel findings, suggesting that PBP4 is a key element in the dissociated phenotype between OXA and FOX and that important gene pathways are associated to the therapeutic response of MRSP to CEF.

Acknowledgements

This research was funded by NIH/NIAID NIH-R56 AI118756 to A.E.R.

Staphylococcus pseudintermedius's PBP4 is Directly Associated with the Dissociated Oxacillin and Cefoxitin Phenotype

Gagetti P[1], Rosato RR[2], Rosato AE[3].

1. Servicio Antimicrobianos, INEI-ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina. 2. Houston Methodist Cancer Center, Houston Methodist Research Institute, Houston, TX.
3. Department of Pathology and Molecular Microbiology Diagnostics-Research, Riverside University Health System, Moreno Valley, CA. pgagetti@anlis.gob.ar

Background

S. pseudintermedius is an important pathogen responsible for infections in dogs and in humans. The emergence and dissemination of methicillin-resistant *S. pseudintermedius* (MRSP) and their multidrug resistance make difficult the treatment of these pathogens. Cefoxitin (FOX) currently utilized to detect *mecA* gene mediated methicillin resistance in *S. aureus* and other staphylococcal species, it is not useful in *S. pseudintermedius*. Given this limitation, in 2016 the CLSI and recently the EUCAST recommended the use of the oxacillin (OXA) disk instead of FOX to detect methicillin resistance in *S. pseudintermedius*. However, the mechanistic bases linked to FOX failure in detecting methicillin resistance in MRSP is still unknown.

Objective

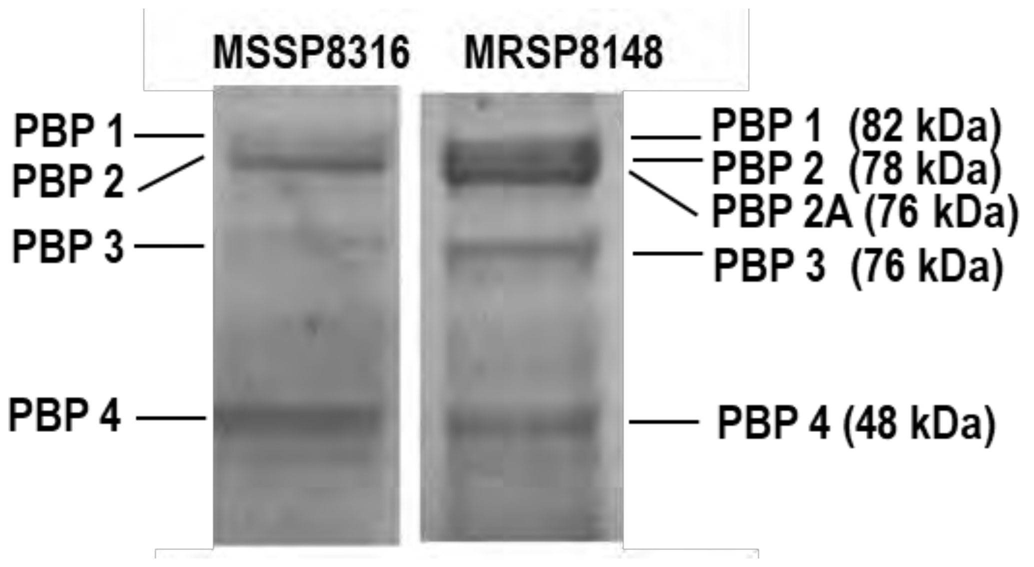
To elucidate the molecular bases of the dissociated phenotype between oxacillin and cefoxitin antibiotics in MRSP

Methods

- MSSP8316 methicillin-susceptible and MRSP8148, MRSP8150 methicillin-resistant strains isolated from canine clinical samples in Argentina, were phenotypically characterized and whole-genome sequenced.
- Membrane abundance, activity of PBPs and competition assays between β-lactams and bocillin were performed.
- PBP sequences were analyzed and compared with reference strains of *S. aureus* and *S. pseudintermedius*.
- RNA-Seq was performed in MRSP8150, MRSP8150 Δ*mecA* and MRSP8150 grown with and without subinhibitory concentrations of cephalexin (CEF). Genome-wide transcript sequencing libraries were prepared according to the manufacturer's recommendations (Illumina, San Diego, CA). Differential gene expression was determined using Lasergene v14 software (DNAStar, Madison, WI), and differences of >3-fold after applying Bonferroni correction were statistically significant.

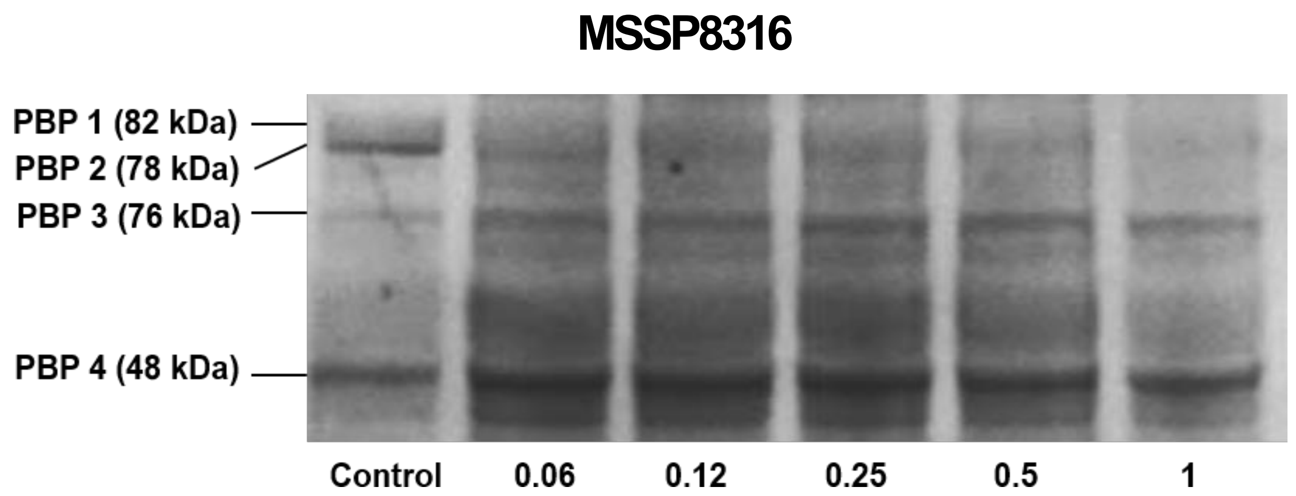
Results

S. pseudintermedius's PBPs

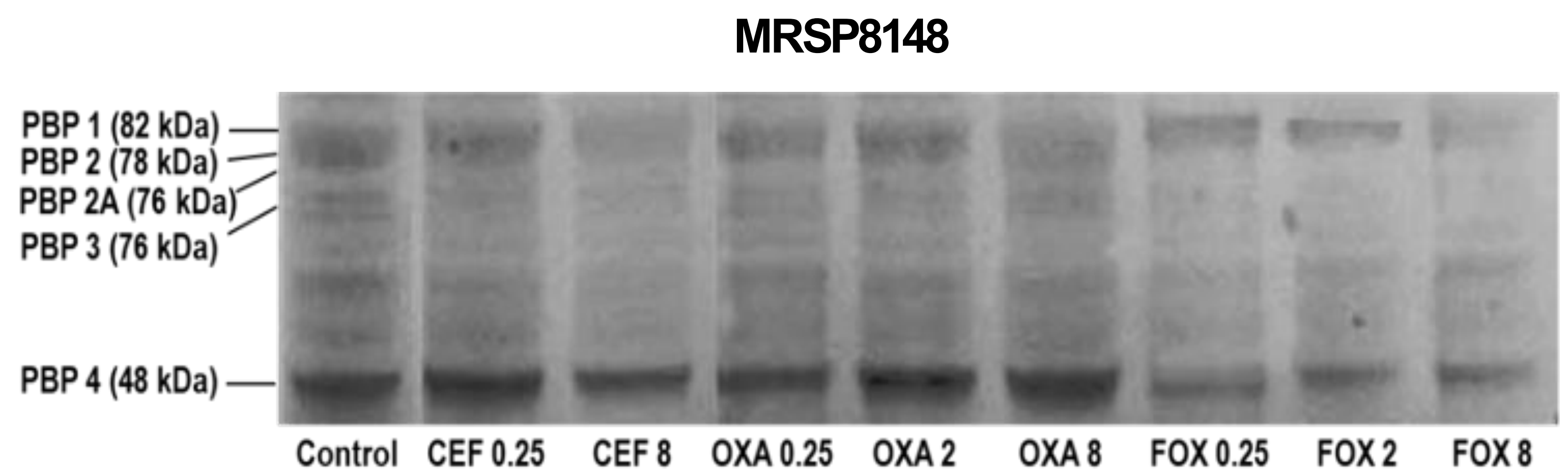


All four native PBPs (1–4) were distinctly identified in membranes' samples from both MSSP and MRSP strains with the presence in the latter of PBP2A, similarly as in MRSA strains.

Competition assays



CEF bounds PBP2 with high affinity.
No changes were seen in PPB1, PBP3, or PBP4.

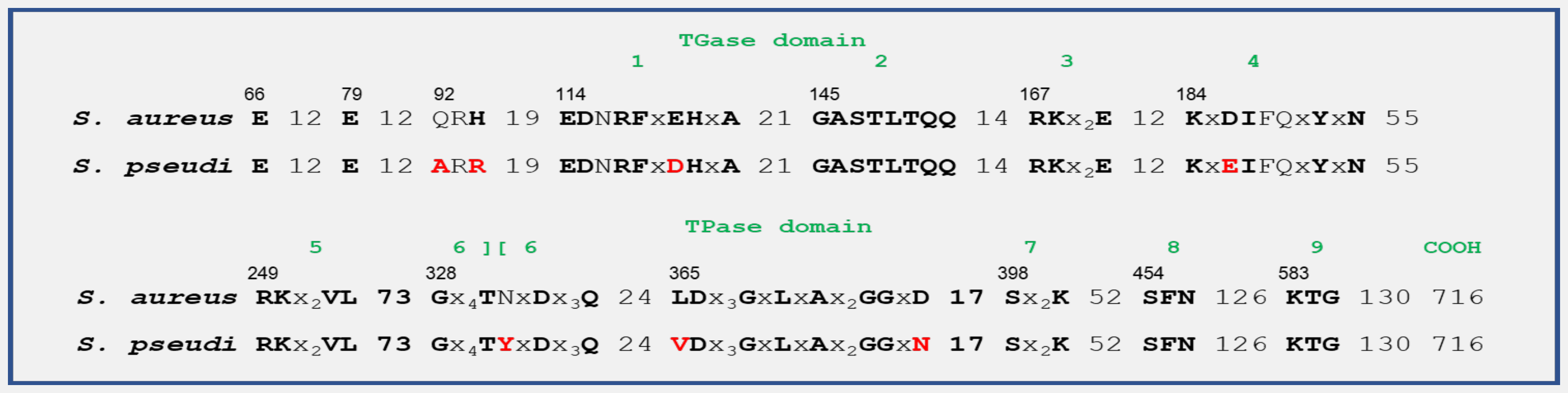


Increased binding of FOX to PBP4 at different concentrations was observed. No changes were observed for CEF and OXA

The observed inhibition of PBP4 by FOX in the competition assays strongly suggests that PBP4 is directly related to the dissociated phenotype between OXA and FOX in MRSP

Analysis of PBP's sequences

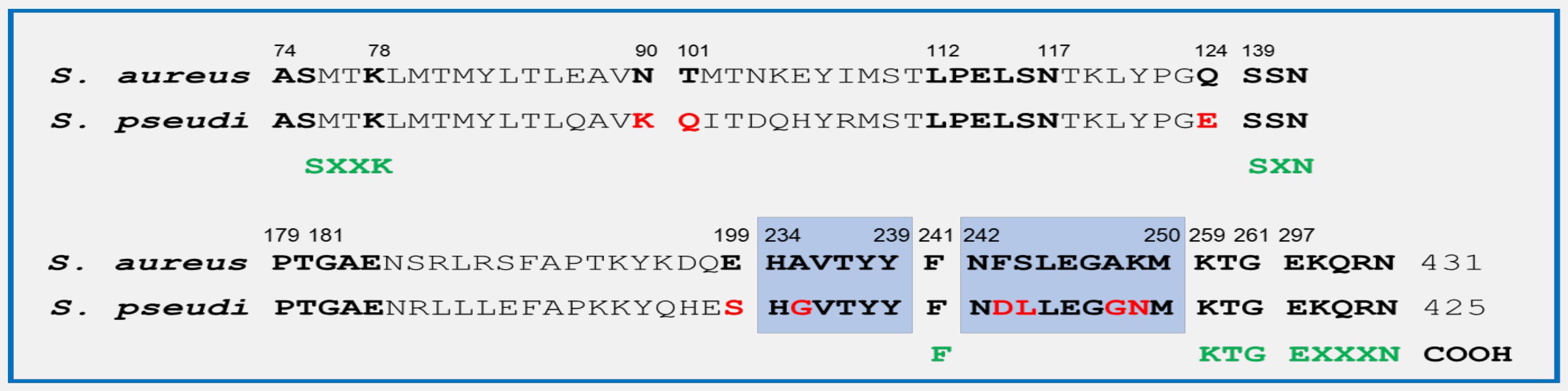
PBP1 is homologous to *S. aureus* PBP2 bifunctional, with transglycosylase and transpeptidase domains. It showed 74% of amino acid identity compared with *S. aureus* N315 PBP2.



It has five aminoacid replacements in motives conserved in class A PBPs responsible for its activity: Q92A, H94R, E120D, D186E, N334Y, L365V and D380N

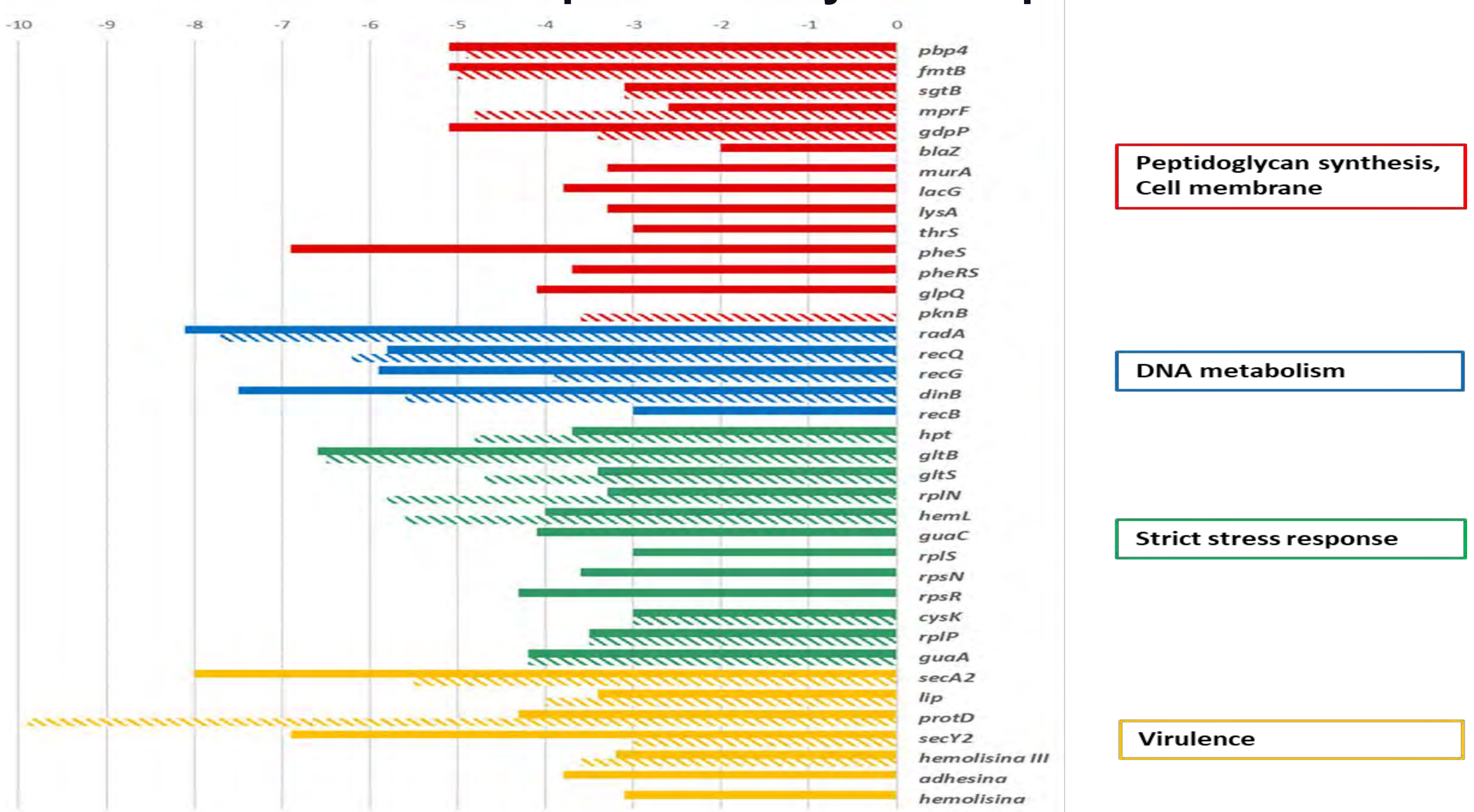
PBP2 and **PBP3** have transpeptidase function and they shared 70 and 74% of aminoacid identity with *S. aureus* N315 counterparts. Moreover, we did not found substitutions in any of the conserved motifs of class B PBPs.

PBP4 has transpeptidase and carboxypeptidase activity and showed 60% of amino acid identity compared with *S. aureus* N315 PBP4 counterpart.



It showed five substitutions in highly conserved regions: A235G, F243D, S244L, A248G and K249N

Gene transcription levels by RNA-Seq



Comparison of MRSP8150 with MRSP8150 Δ*mecA* (full color) and MRSP8150 grown in absence and presence of subinhibitory concentrations of CEF (striped), an antibiotic extensively used to treat MRSP dog infections. Significant difference (>3-fold changes) in the expression of 1189 and 1002 genes respectively was observed.

Conclusions

This study suggest that PBP4 is a key element in the dissociated phenotype between OXA and FOX.

Changes in gene expression were found associated to peptidoglycan synthesis, cell membrane, DNA metabolism and strict stress response could be responsible of the therapeutic failure response of MRSP to CEF. Decrease expression of virulence genes may explain the increase persistence of MRSP infections in dogs that appears directly dependent of the presence of *mecA*.

This research was funded by NIH/NIAID NIH-R56 AI118756 to A.E.R.

Lighting The Viral Dark Matter: Revealing Earth's Cryptic Genosphere Through Environmental Sampling

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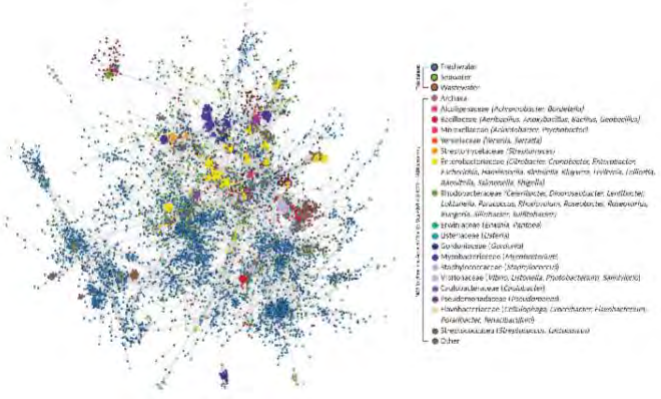
Background: Bacteriophages (or phages) are abundant (estimated 10^{31} particles), diverse (in terms of their genome, morphology, and host), and ubiquitous. However, only a small number of phage genomes have been sequenced and around 60-80% of their sequences do not share homology with previously classified genes. Hence, phages represent the largest repository of undiscovered new biology – what we term as the *cryptic genosphere*. **Goal:** The main goal of this study is to identify novel phage genes, and their corresponding protein function and structure that supplant existing bacterial cell processes, specifically, genes that may overcome oxidative stress and/or repair damaged DNA. **Methods:** Freshwater, seawater, and wastewater samples (~360 liters) were collected around Austin and Houston, TX. Sludge, solids, and contaminants were removed via centrifugation. The viral fraction was recovered and enriched via mixed cellulose esters filter and chemical flocculation, detected with plaque assay and visualized with transmission electron microscopy. Viral DNA was extracted, purified, and sequenced from all samples. Raw reads were analyzed via both the iVirus pipeline and MG-RAST, and several DNA libraries were constructed for functional screening. These DNA libraries were transformed into deletion mutants, Δ*recA* and Δ*catalases*, and either hydrogen peroxide or mitomycin C was added as the stressor for the functional screening. Genes that overcame the stressor and complemented the mutants were analyzed via gel electrophoresis, sequenced, and re-transformed for confirmation. **Results and Conclusion:** We successfully isolated and enriched the viral fraction from all water samples. Freshwater yielded more viral diversity compared to the other two samples, both freshwater and seawater had more diverse functional potential, and ~70 positive hits were obtained from the functional assays. Some of these hits aligned to genes that are involved in DNA repair, potential iron sequestration, and many more.

Acknowledgements: This work was funded in part by the NIH, U.S. Department of Veterans Affairs, MacDonald Foundation, Mike Hogg Foundation, and BCM Seed Funds to AM.



LIGHTING THE VIRAL DARK MATTER: REVEALING EARTH'S CRYPTIC GENOSPHERE THROUGH ENVIRONMENTAL SAMPLING

Carmen Gu Liu[12], James Chang[1], Brianna E. Thompson[3], Lorna Min[4], Kyle E. Weesner[12], Justin R. Clark[12], Sabrina I. Green[12], Austen L. Terwilliger[12], Heidi B. Kaplan[5], Anthony W. Maresso[12]

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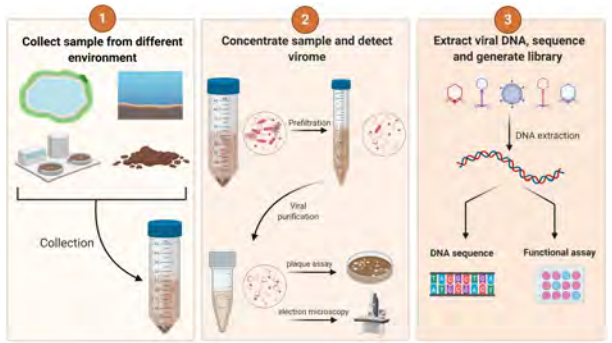
Abstract

Bacteriophages (or phages) are abundant (estimated 10^{31} particles), diverse (in terms of their genome, morphology, and host), and ubiquitous. However, only a small number of phage genomes have been sequenced and around 60-80% of their sequences do not share homology with previously classified genes. Hence, phages represent the largest repository of undiscovered new biology – what we term as the *cryptic genosphere*. The main goal of this study is to identify novel phage genes, and their corresponding protein function and structure that supplant existing bacterial cell processes, specifically, genes that may overcome oxidative stress and/or repair damaged DNA. For this, freshwater, seawater, and wastewater samples (~360 liters) were collected around Austin and Houston, TX. Then, sludge, solids, and contaminants were removed via centrifugation. The viral fraction was recovered and enriched via mixed cellulose esters filter and chemical flocculation, detected with plaque assay and visualized with transmission electron microscopy. Afterwards, viral DNA was extracted, purified, and sequenced from all samples. Raw reads were analyzed via both the iVirus pipeline and MG-RAST, and several DNA libraries were constructed for functional screening. These DNA libraries were transformed into deletion mutants, Δ*recA* and Δ*catalases*, and either hydrogen peroxide or mitomycin C was added as the stressor for the functional screening. Genes that overcame the stressor and complemented the mutants were analyzed via gel electrophoresis, sequenced, and re-transformed for confirmation. Overall, we successfully isolated and enriched the viral fraction from all water samples. Freshwater has more viral diversity compared to the other two; both freshwater and seawater have a more diverse functional potential; and ~70 positive hits were obtained from the functional assays. Sanger sequencing showed that some of these hits aligned to genes that are involved in DNA repair and potential iron sequestration.

Overview



Methods

Table 1. Environmental sample collection. Various amount of freshwater, seawater and wastewater samples were collected around Texas, USA

Samples	Source type	Volume and/or amount processed	Source City
Freshwater	Creek	57 L	Austin, TX
	River	57 L	Austin, TX
	Lake	57 L	Houston, TX
	Wetland	57 L	Houston, TX
Seawater	Beach	57 L	Galveston, TX
	Beach	57 L	La Porte, TX
Wastewater	WWTP	9.5 L	Austin, TX
	WWTP	6.6 L	Houston, TX

Figure 1. Collection sites for freshwater samples

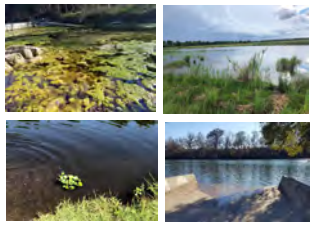


Figure 2. Concentration of viral fraction. (a) To recover the viral fraction of environmental samples, different processing routes were employed, which depends on the volume and the type of the sample. For freshwater and seawater, chemical flocculation via iron chloride and resuspension with citrate buffer was performed. For wastewater, filtration via MCE (mixed cellulose esthers) membranes and resuspension with NaOH and H2SO4 was employed. **(b)** Different stages of freshwater and seawater processing. **(c)** Different stages of wastewater processing

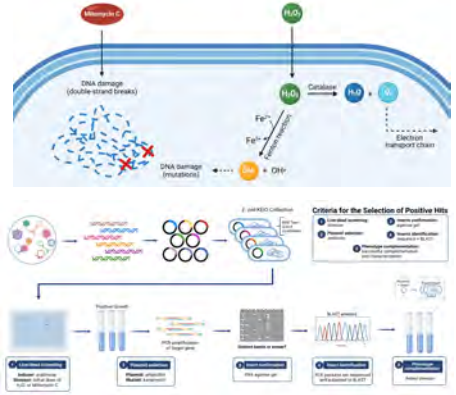


Figure 3. Functional screening and criteria for the selection of positive hits. DNA libraries were constructed from environmental DNA and transformed into mutant *E. coli*. Stressors (Mitomycin C or H2O2) were used to screen for hits that will overcome either the oxidative stress or DNA damage. Positive hits were analyzed, sequenced, and confirmed

Results

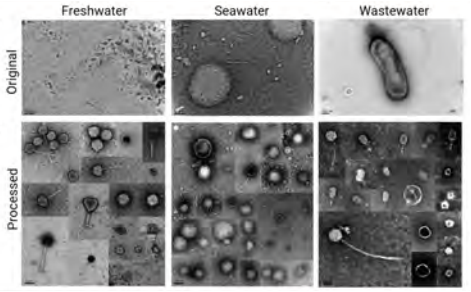


Figure 4. Transmission electron microscopy (TEM) imaging of all viromes. To visualize the viral diversity, TEM was performed on both the original and processed/enriched viral samples. More viral-like-particles (VLPs) were found in the processed samples

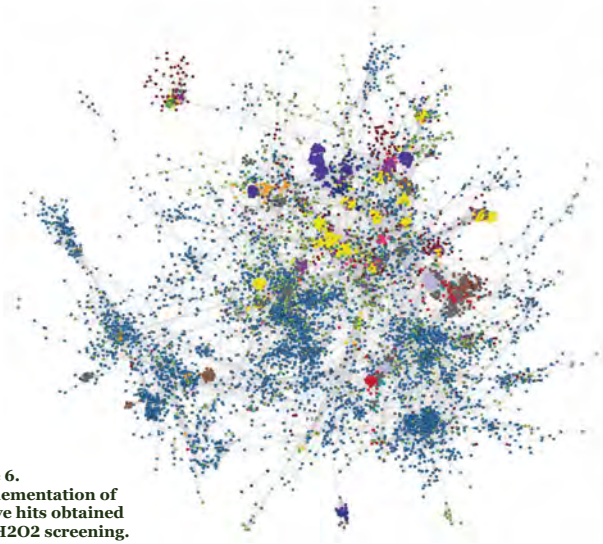


Figure 5. Taxonomical analysis of all viromes. Viral contigs were identified with VIBRANT and these contigs were clustered with vCONTACT2 based on their protein cluster profile. Each node represents a viral genome and is colored based on the putative host family. Freshwater viral contigs were colored in blue, seawater in green and wastewater in brown. Small clusters and singletons are not shown here

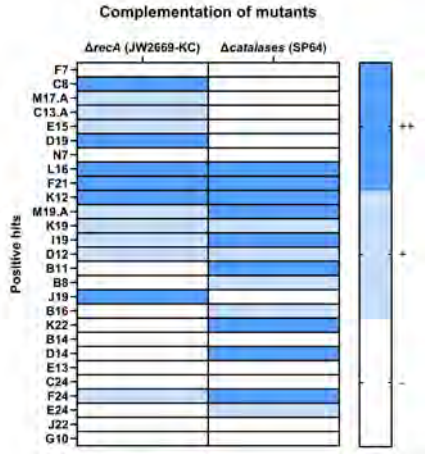
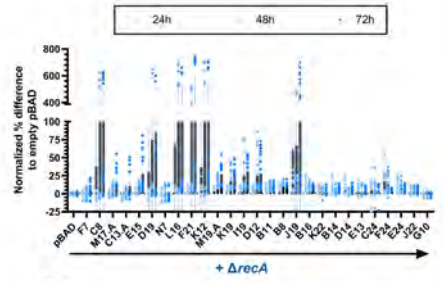


Figure 6. Complementation of positive hits obtained from H2O2 screening. Plasmids were extracted from the hits and re-transformed into Δ*recA* and Δ*catalases*. These transformants were treated with lethal doses of H2O2 for 72 hours in 5 mL cultures. Absorbance(OD600nm) was measured every 24 hours and data was normalized to the mutants that contain empty pBAD. Successful complementation was defined as >50% change



Conclusions

- Metagenomic sequencing data showed distinct viromes based on the different samples collected from different environment (freshwater, seawater, and wastewater) and each of these viromes have a lot of functional potential (i.e. they encode genes that are involved in multiple metabolic and functional pathways)
- Screenings showed positive hits that complemented bacterium lacking genes in DNA repair (*recA*) or detoxification system (*ahpF, ahpC, katG, katE)*, which suggests that enriched viromes encode potential genes that aid in their survival of oxidative stress

Future Directions

- Characterization of genes isolated from functional screening (measurement of H2O2 production and DNA damage)
- Pick one to three genes to further study in detail (function and structure)

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Mechanism of Carbapenem Resistance in Pseudomonas aeruginosa Cystic Fibrosis Isolates

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Background: *Pseudomonas aeruginosa* is a Gram-negative, multidrug-resistant pathogen that causes life-threatening infections in immunocompromised patients. While pseudomonal infections are commonly treated with beta-lactams (e.g.: cephalosporins) and/or aminoglycosides (e.g.: tobramycin), the rise of extensively drug-resistant (XDR) strains is rendering these therapeutics obsolete. One class of drugs reserved for XDR infections is carbapenems. Unfortunately, in a recent survey of multidrug-resistant *P. aeruginosa* strains isolated from pediatric cystic fibrosis (CF) patients, we demonstrated that a substantial fraction of isolates is already resistant to carbapenems such as imipenem and/or meropenem.

Hypothesis/Goals: We aimed to elucidate the mechanism of carbapenem resistance in drug-resistant *P. aeruginosa* CF isolates.

Methods: We performed whole-genome sequence analysis in various carbapenem-resistant and susceptible strains and computed average nucleotide identity (ANI) scores to identify closely related genomes. Using this method, we identified two pairs of carbapenem-resistant and susceptible strains with ANI scores above 99.9%. To determine the genetic basis of carbapenem resistance, we aligned these genomes using a mutation identification tool *breseq*.

Conclusions: *In silico* analysis suggests that carbapenem resistance in one *P. aeruginosa* CF isolate was conferred by a mutation in the outer membrane porin OprD and peptidoglycan recycling enzyme Mpl (involved in the regulation of the cephalosporinase AmpC). Consistent with the hypothesis that AmpC is overexpressed in this strain, vaborbactam had a modest but significant effect in increasing the pathogen's susceptibility to meropenem. In another CF isolate, carbapenem resistance was likely conferred by a XerD recombinase-dependent integration of an OXA-2 family class D beta-lactamase.

Acknowledgements: This work was supported by the Cystic Fibrosis Foundation grant KANG21H0 awarded to Donghoon Kang.

Mechanism of Carbapenem Resistance in *Pseudomonas aeruginosa* Cystic Fibrosis Isolates

Donghoon Kang and Natalia V. Kirienko

Department of BioSciences, Rice University, Houston, TX

Abstract

Background: *Pseudomonas aeruginosa* is a Gram-negative, multidrug-resistant pathogen that causes life-threatening infections in immunocompromised patients. While pseudomonal infections are commonly treated with beta-lactams (e.g.: cephalosporins) and/or aminoglycosides (e.g.: tobramycin), the rise of extensively drug-resistant (XDR) strains is rendering these therapeutics obsolete. One class of drugs reserved for XDR infections is carbapenems. Unfortunately, in a recent survey of multidrug-resistant *P. aeruginosa* strains isolated from pediatric cystic fibrosis (CF) patients, we demonstrated that a substantial fraction of isolates is already resistant to carbapenems such as imipenem and/or meropenem.

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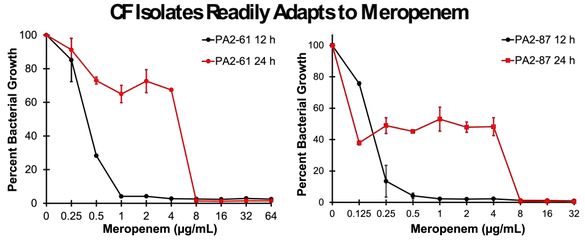
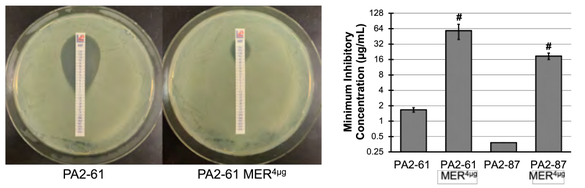
oprD Mutation Drives Meropenem Resistance in *P. aeruginosa* CF Isolates

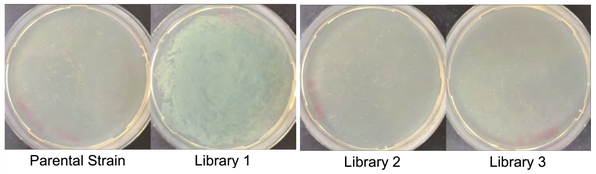
CF Isolates Readily Adapts to Meropenem



Meropenem-Adapted Bacteria are *bona fide* Resistant Mutants



Transposon Mutagenesis of Drug-Susceptible Isolate PA2-87



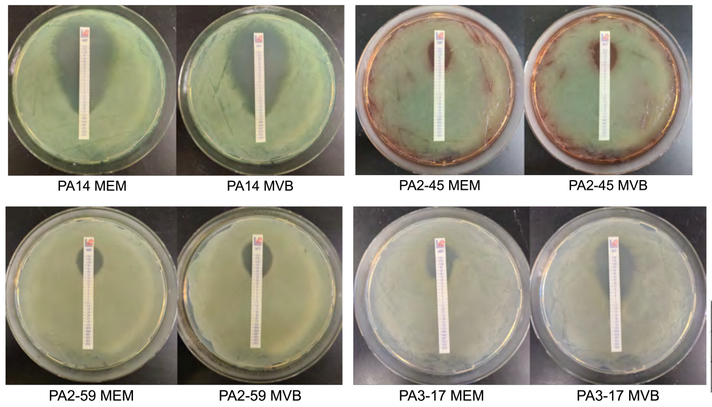
Each library consisted of ~3000 transposon mutants and selected on LB + 4 μg/mL meropenem. Resistant colonies in Library 1 were sequenced by arbitrary PCR. **Transposon insertion was in porin *oprD*.**

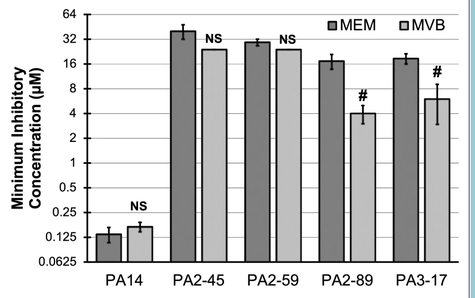
Mutations in Meropenem-Adapted CF Isolates

Strain	Gene	Function	Mutations
PA2-61	*oprD*	Basic amino acid, basic peptide and imipenem outer membrane porin OprD precursor	Δ2bp (630-631/1326 nt)
PA2-87	*oprD*	Basic amino acid, basic peptide and imipenem outer membrane porin OprD precursor	Δ2bp (800-801/1326 nt)

Mechanism of Carbapenem Resistance in Drug-Resistant Isolates

Inhibition of Carbapenemase via Vaborbactam



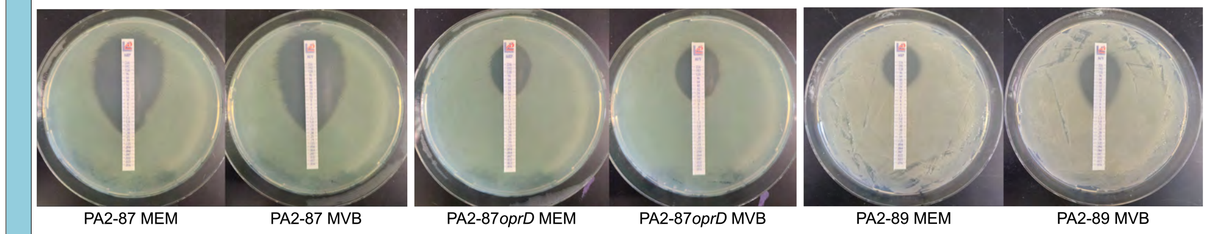


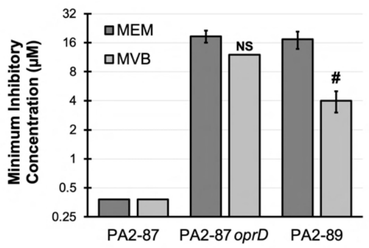
	PA14	PA2-45	PA2-59	PA2-89	PA3-17	PA5-40	ST773
MEM	S	R	R	R	R	R	R
MVB	S	R	R	I	I	R	R

Vaborbactam Increases Meropenem Susceptibility in an Isolate Carrying a *mpl* Nonsense Mutation

	AMK	AMP	SAM	ATM	CFZ	FEP	FOX	CAZ	CRO	CIP	ETP	GEN	IPM	LVX	MEM	NIT	TZP	TET	TOB	SXT
PA2-87	S	R	R	R	R	I	R	S	R	R	R	S		R	S	R	I	R	S	R
PA2-89	R			S		R		R		R		I	R		R		I		S	

PA2-87 and PA2-89 are very closely related strains. They have an average nucleotide identity (ANI) score of 99.97% corresponding to ~300 SNPs between their genomes.



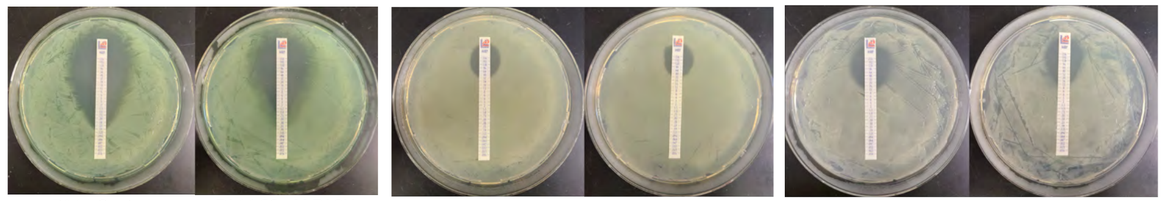


Polymorphisms Relevant to Carbapenem Resistance

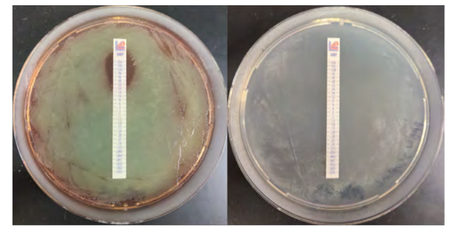
Gene	Function	PA2-87	PA2-89
mpl	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase		+1 bp (111/1356 nt)
oprD	Basic amino acid, basic peptide and imipenem outer membrane porin OprD precursor		Δ1 bp (1143/1326 nt)
mexR	MarR family transcriptional regulator MexR	+39727 bp	

mexR mutation has been shown to cause overexpression of the *mexAB-oprM* multidrug efflux system. *mpl* mutation has been shown to cause overexpression of the cephalosporinase *ampC*.

Efflux Pump Inhibitor PAβN Does Not Affect Meropenem Susceptibility in CF Isolates

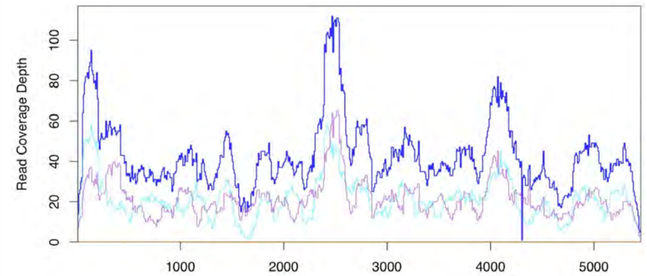


Identification of an Isolate Highly Susceptible to PAβN

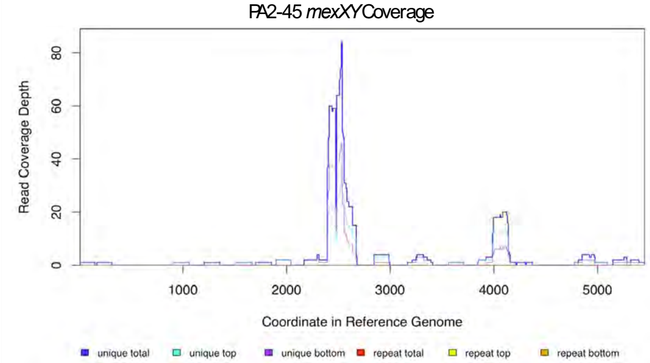


Possible Explanation: PA2-45 Lacks MexXY Efflux System

PA14 *mexXY* Coverage



PA2-45 *mexXY* Coverage



Conclusions

- *Pseudomonas aeruginosa* CF isolates can readily adapt to meropenem via mutations in the outer membrane porin OprD
- Vaborbactam has a modest effect on meropenem susceptibility in certain drug-resistant CF isolates
- In one resistant isolate carrying a *mpl* nonsense mutation, vaborbactam likely attenuates AmpC activity
- Multidrug efflux pump inhibitor PAβN did not affect meropenem susceptibility in drug-resistant isolates
- PAβN substantially inhibited bacterial growth in one isolate, PA2-45, which lacked the MexXY efflux system

This work was supported by the Cystic Fibrosis Foundation grant KANG21H0 awarded to Donghoon Kang

Oral Antibiotic Stepdown Therapy for Uncomplicated Streptococcal Bloodstream Infections

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Background: Streptococcal bloodstream infections (BSIs) can result in serious complications including sepsis. Although most BSIs are managed with intravenous (IV) antibiotics, there is growing literature supporting oral antibiotics in various infections, including uncomplicated BSIs. Advantages to oral therapies include shortened hospital length of stay, reduced treatment costs, and avoidance of line related complications. Currently, there is a paucity of data supporting the use of oral antibiotics in streptococcal BSIs.

Goals: The goal of this study is to determine if using oral antibiotic as a stepdown regimen is as efficacious as a full course of IV antibiotics for the treatment of uncomplicated streptococcal BSIs.

Methods: This retrospective cohort study compared patients who received a full treatment course of IV antibiotics verses completion of antibiotic therapy with an oral stepdown regimen for uncomplicated streptococcal BSIs at a large county hospital from September 2019 to September 2021. Patients 18 and older were included if they had at least one positive blood culture for any *Streptococcus* species and excluded if they received antimicrobial treatment for ≥ 16 days, had a polymicrobial BSI or a complicated BSI such as endocarditis, osteomyelitis, or meningitis. The primary endpoint was clinical failure, which was a composite endpoint defined as BSI recurrence with the same pathogen and infection-related readmission within 30 days from completion of antibiotics. Multivariate logistic regression of the primary end point will be conducted, and adjusted odds ratios will be calculated to control for significant univariate characteristics.

Results: Data collection and analysis are ongoing. Preliminary results will be examined and completed by January 2022.

Conclusions: The results from this study will provide additional data regarding the role of oral antibiotics in the treatment of uncomplicated streptococcal BSIs. Utilizing oral antibiotics will help reduce collateral damage from prolonged IV regimens and optimize antimicrobial stewardship efforts.

Oral Antibiotic Stepdown Therapy for Uncomplicated Streptococcal Bloodstream Infections

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Parkland Health & Hospital System, Dallas, TX

BACKGROUND

Streptococcal bloodstream infections (BSIs) can result in serious complications including sepsis.[1] Although most BSIs are managed with intravenous (IV) antibiotics, there is growing literature supporting the use of oral antibiotics in various infections, including uncomplicated BSIs.[2] Advantages to oral therapies include shortened hospital length of stay, reduced treatment costs, and avoidance of indwelling line related complications.[3] Currently, there is a paucity of data supporting the use of oral antibiotics in streptococcal BSIs.

PURPOSE

To determine if an oral antibiotic stepdown regimen is as efficacious as a full course of IV antibiotics for the treatment of uncomplicated streptococcal BSIs.

METHODS

Study Design: Retrospective cohort study comparing the efficacy of IV antibiotics for the full course of therapy versus a regimen completed with oral stepdown therapy for uncomplicated streptococcal BSIs.

Study Population: Patients with uncomplicated streptococcal BSIs at a large county hospital from September 2019 to September 2021.

Inclusion Criteria

- Adults ≥ 18 years of age
- At least one positive blood culture for any *Streptococcus* species deemed a true pathogen

Exclusion Criteria

- Antimicrobial treatment ≥ 16 days
- Positive blood culture after index blood culture
- Polymicrobial BSIs
- Complicated BSI: endovascular or metastatic focus of infection, intravascular prosthetic device, undrained abscess >5 cm, deep tissue involvement, extensive burns, CNS infection, bone and joint infection

Primary Outcome

- Clinical failure defined as a composite of BSI recurrence with the same pathogen or infection-related readmission within 30 days from antibiotic completion

Secondary Outcomes

- All cause mortality 30 days from antibiotic completion
- Hospital length of stay

RESULTS

Figure 1: Patient Inclusion

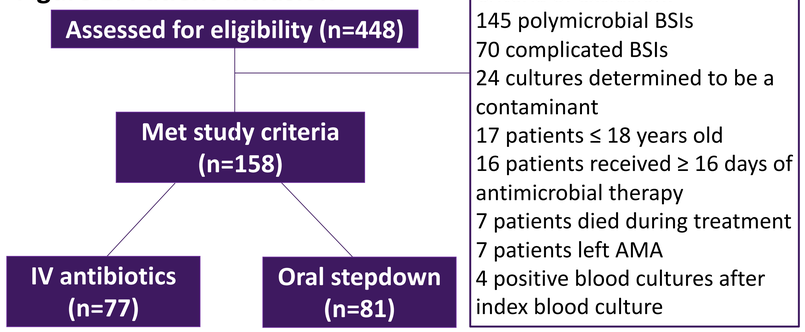


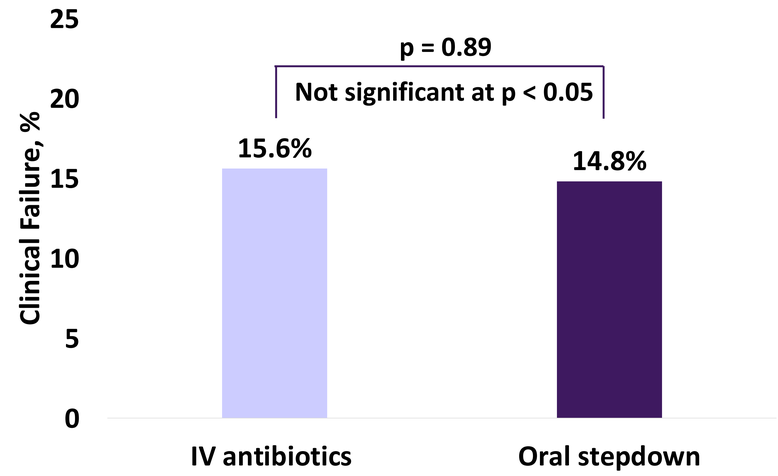
Table 1: Characteristics at Baseline

Characteristic	IV antibiotics (n=77)	Oral stepdown (n=81)
Age, y, median (IQR)	53 (43-64)	54 (43-62)
Male sex, n (%)	59 (77)	55 (68)
Diabetes mellitus, n (%)	31 (40)	36 (44)
Cirrhosis, n (%)	20 (26)	12 (15)
Immunocompromised, n (%)	12 (16)	14 (17)
Main source of infection, n (%)		
Skin and soft tissue	26 (34)	44 (54)
Line-related	4 (5)	1 (1)
Intra-abdominal	20 (26)	8 (10)
Respiratory	5 (7)	5 (6)
Odontogenic	8 (10)	7 (8)
Genitourinary	4 (5)	5 (6)
Unknown	10 (13)	12 (15)
Active empiric therapy, n (%)	77 (100)	81 (100)
Time to oral stepdown, d, median (IQR)	--	5 (3.5-7.0)
Total duration, d, median (IQR)	14 (8.0-14.0)	14 (14.0-14.5)

Table 2: Primary and Secondary Outcomes

Outcome	IV antibiotics, No. of patients/ total (%)	Oral stepdown, No. of patients/ total (%)	*p*-value
Primary endpoint			
Clinical failure	12/77 (15.6)	12/81 (14.8)	0.89
BSI recurrence	1/77 (1.3)	0/81 (0.0)	
Infection related readmission	11/77 (14.3)	12/81 (14.8)	0.92
Secondary endpoints			
All-cause mortality	2/77 (2.6)	0/81 (0.0)	
Hospital length of stay, d, median (IQR)	12 (6.5-19.5)	6 (4.0-9.0)	

Figure 2: Primary Outcome – Clinical Failure



CONCLUSION

In this retrospective study with ongoing analysis, there was no difference in clinical outcomes between those who completed the full course with IV antibiotics compared to those who completed therapy with an oral antibiotic stepdown regimen for uncomplicated streptococcal BSIs.

The results of this study are hypothesis generating and larger randomized controlled trials are needed to confirm these findings.

LIMITATIONS

- Retrospective design
- Unable to assess adherence post hospital discharge
- Clinical failure undetected if patient accessed care at an outside facility
- Study results not applicable to complicated BSIs
- Further studies needed to determine optimal timing to oral antibiotic stepdown

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Mutational Switch-Backs Can Accelerate Evolution of Francisella to a Combination of Ciprofloxacin and Doxycycline

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Abstract:

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 evolutionary outcome of adapting the Live Vaccine Strain (LVS) of *Francisella* to two non-interacting drugs, ciprofloxacin and doxycycline, individually, sequentially and in combination.

Results: Despite their individual efficacies and independence of mechanisms, evolution to the combination appeared to progress faster than evolution to the two drugs sequentially. We conducted a longitudinal mutational analysis of the populations evolving to the drug combination and by genetically reconstructing the identified evolutionary pathway and biochemical validation, we discovered that after the appearance of an initial weak generalist mutation (FupA/B), each successive mutation was associated with adaptation to one drug or the other, and in combination, these mutations allowed the population to more efficiently ascend the fitness peak through a series of evolutionary switch-backs. Clonal interference, weak pleiotropy and positive epistasis also contributed to combinatorial evolution.

Conclusions: This finding suggests that under some selection conditions, the use of non-interacting drug pairs as a treatment strategy may result in a more rapid ascent to multi-drug resistance and serves as a cautionary tale. We are currently studying evolution of this intracellular pathogen to the two drug combination inside a macrophage environment. This will allow us to study the role of mutational switch-backs during intracellular infection and determine if heterogeneity in drug concentrations across mammalian cells can contribute to this phenomenon.

Acknowledgements: This work is supported by funds from the Defense Threat Reduction Agency (grant HDTRA1-15-1-0069) to Y.S. The content of the information in this paper does not necessarily reflect the position or the policy of the federal government, and no official endorsement should be inferred.

Mutational Switch-Backs Can Accelerate Evolution Of *Francisella* To A Combination Of Ciprofloxacin And Doxycycline

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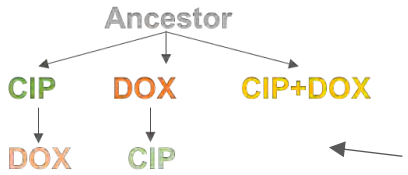
(1) *F. tularensis* Live Vaccine Strain (LVS) was propagated in the presence of increasing concentrations of either CIP, DOX or both drugs. Mono-evolved populations were further evolved to the second drug.

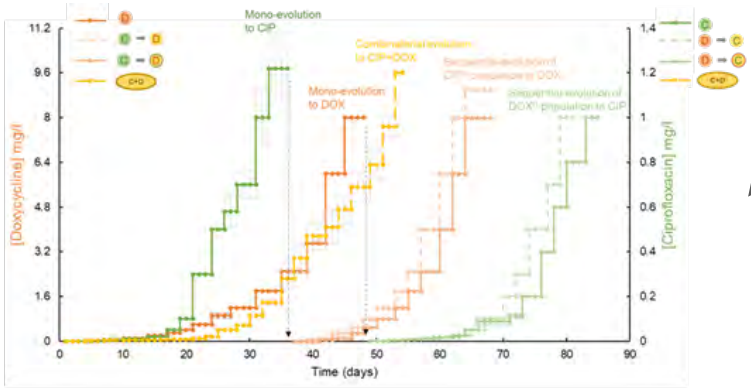


Introduction

- Continuing rise of drug resistant pathogens
- Polypharmocological approaches
- Knowledge of effective drug combinations and their evolutionary interactions

***Francisella* and treatment for tularemia**

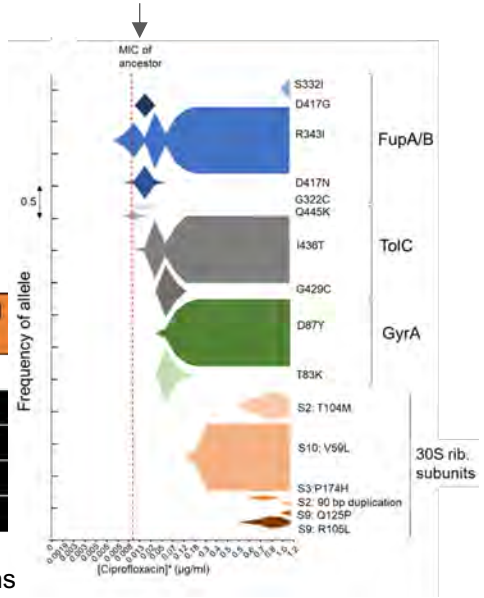
- Category A bioterrorism agent
- CDC maintains stockpiles of Ciprofloxacin (CIP) and Doxycycline (DOX) in case of mass casualty situation
- What is the efficacy of administering two drugs vs either one?
- Does combination treatment delay the onset of resistance?



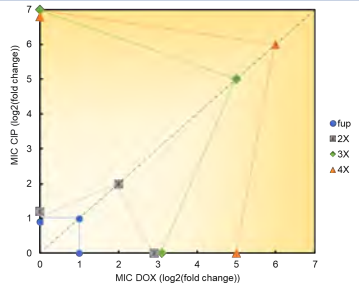
(2) Timeline of evolution under different selection environments showed that the drug combination (yellow curve) was unable to extend the time taken by cells to acquire resistance.

	Selecting agent	FupA/B	AcrAB-TolC	Gyrases/topoisomerase IV	30S ribosomal subunits
Mono-selection	CIP	+		+	
	DOX	+	+		+
Sequential-selection	CIP^R→DOX	+	+	+	+
	DOX^R→CIP	+	+	+	+
Combination	CIP+DOX	+	+	+	+

(3) Genomic analysis of final evolved populations identified 4 classes of mutations involved in resistance. While mutations in FupA/B appeared to have a generalist role in CIP and DOX resistance, other mutations were specific to one drug or the other. Sequential and combinatorially evolved populations showed mutations in all 4 classes.



(4) Longitudinal metagenomic analysis identified the order of appearance of the mutations and the presence of clonal interference



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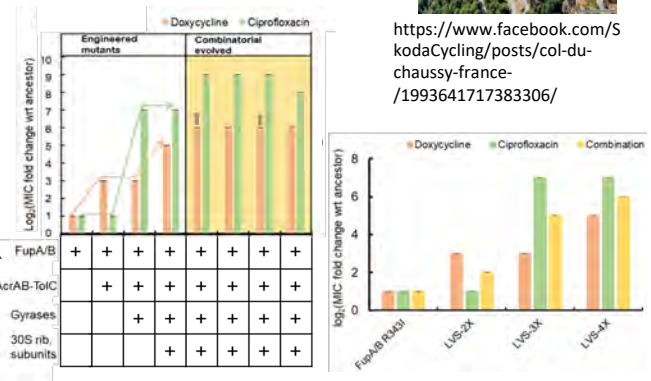
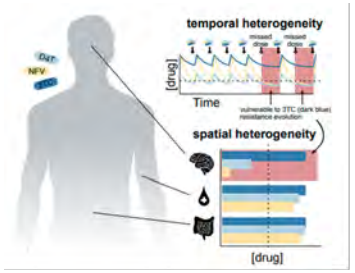
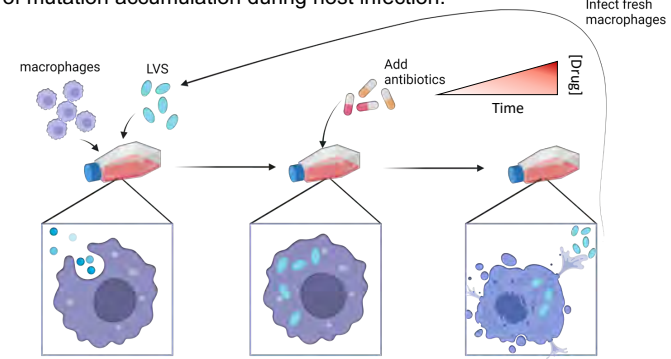


(5) Re-creating mutant genotypes in the order in which they appeared during evolution helped identify a mechanism of mutational switchbacks that contributed to accelerated evolution to the drug combination. After the occurrence of a weak generalist mutation, successive mutations accumulated in a manner such that they provided resistance to one drug or the other. This allowed the cells to steadily climb the fitness landscape without the need for simultaneous acquisition of multiple mutations.

(6) Switchbacks could play a role during *in vivo* evolution of this intracellular pathogen. Experimental evolution of LVS to the drug combination inside macrophages will provide interesting insight into dynamics of mutation accumulation during host infection.





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Bacteriophage-Containing Biodegradable Microsphere Technology to Treat Osteomyelitis

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Background: The rise in antimicrobial resistant (AMR) infections is a growing concern within healthcare and scientific communities. With few antibiotics in the pipeline and growing antibiotic resistance, bacteriophage (phage) provide a unique biological approach to the AMR problem. Several issues have been raised concerning the ability to deliver active bacteriophages using degradable drug delivery systems. In manufacturing poly(lactic-co-glycolic acid) (PLGA) microspheres, the two steps that present the greatest possibility for phage inactivation are contact with the organic solvent dichloromethane and lyophilization.

Goals: As some studies have shown lyophilization is less likely to result in a significant loss of phage lytic activity, in this study we investigated two protocols for microsphere manufacture, utilizing our previously developed microsphere technology used to deliver antibiotics, to focus on the exposure to dichloromethane.

Methods: Two bacteriophage, phage K and SA4, were tested for their ability to lyse a clinical *Staphylococcus aureus* isolate, known as UAMS-1 or ATCC 49230, obtained from an osteomyelitis infection. Both phage demonstrated lytic activity against UAMS-1 *S. aureus* grown on agar plates, in liquid culture, and in biofilms. The PLGA microspheres were manufactured according to a water-oil-water protocol that yields 250 mg of microspheres. Two methods were evaluated. The first included adding a 2.5 x 10^{10} phage/ml solution directly to a PLGA-dichloromethane mixture (Method 1). Contact time between the phage and the solvent was reduced by using 2-propanol to precipitate the microspheres. In the second method, the 2.5 x 10^{10} phage/ml solution was added to a polyvinyl alcohol (PVA) solution before adding this mixture to the PLGA-dichloromethane solution (Method 2). Both methods were evaluated using an elution assay, and the eluent was collected after 24 hr, 72 hr, and 7 days. Each eluent was spotted onto a lawn of UAMS-1 *S. aureus*, and the resulting plaque forming units (PFUs) were counted. The total phage eluted over the 7-day period was calculated, as was the average entrapment efficiency, for the 250 mg batches. SPSS software was used for data analysis and a t-test was performed to compare the data for the 4 trials completed per method.

Results: For Method 1, a total of 2.0 x 10^{7} phage eluted after 7 days, yielding a 0.6% entrapment efficiency. For Method 2, a total of 5.1 x 10^{5} phage eluted after 7 days, yielding a 0.02% entrapment efficiency. The t-test results revealed a statistically significant difference between the two protocols ($p = 0.012$).

Conclusion: In this *in vitro* study, we compared two methods to manufacture phage-containing PLGA microspheres to determine which method resulted in the greatest elution of active phage and entrapment efficiency. Method 1 eluted a statistically greater number of active phage and had a greater entrapment efficiency. These results indicate that further studies should be performed to determine the effectiveness of phage-containing PLGA microspheres against *S. aureus* in *in vitro* biofilms and in an *in vivo* animal model.

Acknowledgments: We thank Dr. Danielle Garsin for use of the CSLM, Drs. Anthony Maresso and Austen Terwilliger, TAILΦR Labs, Baylor College of Medicine for suppling the phage, and the Dean's Office at McGovern Medical School for financial support.

Bacteriophage-containing Biodegradable Microsphere Technology to Treat Osteomyelitis

[1] Analisa J. Narro; [2] Erin Brown; [1] Heidi B. Kaplan; [1] Catherine G. Ambrose
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Introduction

The increasing incidence of antimicrobial resistant (AMR) infections is a growing concern within healthcare and scientific communities. Although the Centers for Disease Control and Prevention reports more than 2.8 million AMR infections annually in the U.S. resulting in more than 35,000 deaths, only a limited number of new antibiotics are in the pharmaceutical pipeline. Researchers have responded by developing alternative antimicrobial strategies. The utilization of bacteriophage to target and kill bacteria within the site of infection offers a biological approach to combat the AMR problem. Currently, no bacteriophage delivery systems are being used to target musculoskeletal infections. Several issues have been raised concerning to the ability to manufacture active bacteriophages in degradable drug delivery systems.[1] In our process of manufacturing poly(lactic-co-glycolic acid) (PLGA) microspheres, the two steps that present the greatest possibility for bacteriophage inactivation are the contact with the organic solvent dichloromethane (DCM) and lyophilization. Interestingly, studies have shown that lyophilization is less likely to result in a significant loss of phage lytic activity.[2] As a result, in this work we investigated two protocols for microsphere manufacture, building upon our previously developed microsphere technology used to deliver antibiotics[3], focusing on reducing the contact time of the bacteriophage with dichloromethane.

Materials and Methods

We tested two bacteriophages, phages K and SA4 (Fig. 1), for their ability to lyse a clinical *Staphylococcus aureus* isolate, known as UAMS-1 or ATCC 49230, obtained from an osteomyelitis infection. Both bacteriophages demonstrated lytic activity against UAMS-1 *S. aureus* grown on agar plates, in liquid culture, and in biofilms (Fig. 2). Phage K was used in the microsphere formulations.

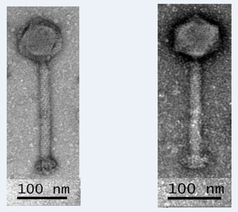

Figure 1. Transmission electron microscope images of the two phage used, K and SA4.

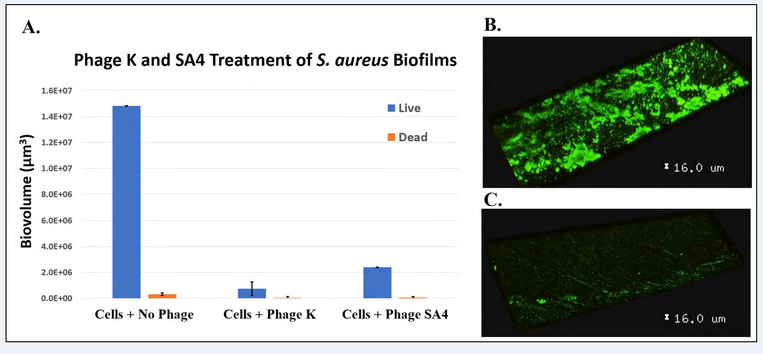

Figure 2. Bacteriophage treatment of *S. aureus* biofilms. *S. aureus* strain UAMS-1 4-day biofilms grown on 5 mm diameter polymethyl-methacrylate discs in synthetic interstitial fluid in 24-well plates at 37°C with daily medium changes were treated for 8 hr with 10^8 phage K or phage SA4. The biofilm discs were stained using a LIVE/DEAD BacLight Bacterial Viability Kit (ThermoScientific) for 12 min at room temperature and imaged with a Fluoview FV3000 confocal laser scanning microscope (CLSM) (Olympus). The images of treated and untreated biofilms were analyzed with ImageJ. A) The biovolumes shown for untreated biofilms or biofilms treated with phages K or SA4 reflect the green ('live' intact cells) or red ('dead' permeable cells) fluorescence of each biofilm. Biovolume is equivalent to cell number, as 1 cell is roughly 1 µm³. B) A CLSM image of an untreated 4-day *S. aureus* biofilm. C) A CLSM image of a 4-day *S. aureus* biofilm treated with phage K for 8 hr.

The PLGA microspheres were manufactured according to a water-oil-water protocol that yields 250 mg of microspheres.[3] Two different methods were evaluated. The first method included adding a 2.5 x 10^{10} phage/ml solution directly to a PLGA-dichloromethane mixture (Method 1). The contact time between the bacteriophage and the solvent was reduced by using 2-propanol to precipitate the microspheres. The second method included an additional protocol modification in which the 2.5 x 10^{10} phage/ml solution was added to a polyvinyl alcohol (PVA) solution before adding this mixture to the PLGA-dichloromethane solution (Method 2). Images of the main procedural steps can be seen in Figure 3.

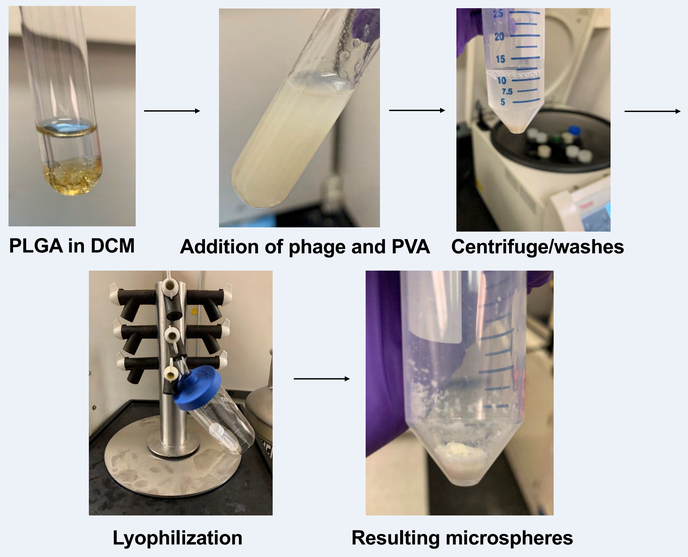

Figure 3. Production of the bacteriophage-containing PLGA microspheres. The microspheres were generated using a water-oil-water technique.[3] The images depict the core steps in the microsphere production protocol.

Both methods were evaluated using an elution assay in which 25 mg of lyophilized microspheres were added to 1 ml of phosphate buffered saline (PBS) in a glass test tube at 37°C, and the eluent was collected at 24 hr, 72 hr, and 7 days. Ten microliters of each eluent were spotted in triplicate onto a lawn of UAMS-1 *S. aureus*, incubated overnight at 37°C and the resulting plaque forming units (PFUs) were counted. The total phage eluted over the 7-day period was calculated, as was the average entrapment efficiency for the 250 mg batches. The size distribution of the bacteriophage-containing microspheres was also determined using a dissecting microscope (Fig. 4). SPSS software was used for data analysis and a t-test was performed to compare the data for the 4 trials completed per method.

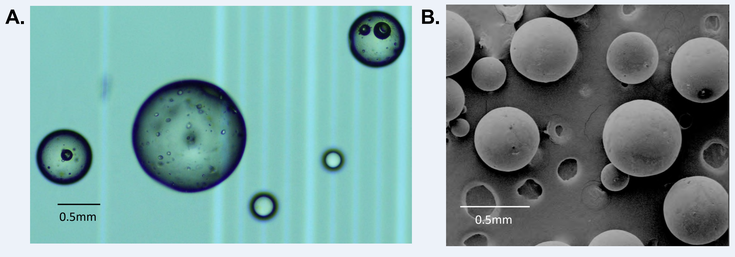

Figure 4. A) Light microscopy images and B) Scanning electron microscopy images of the bacteriophage-containing poly(lactic-co-glycolic acid) microspheres.

Results

For Method 1, an average of 2.0 x 10^7 phage eluted after the first 7 days (Fig. 5), yielding a 0.6% entrapment efficiency. For Method 2, an average of 5.1 x 10^5 phage eluted after the first 7 days (Fig. 5), yielding a 0.02% entrapment efficiency. The t-test results revealed a statistically significant difference between the protocols (p= 0.012).

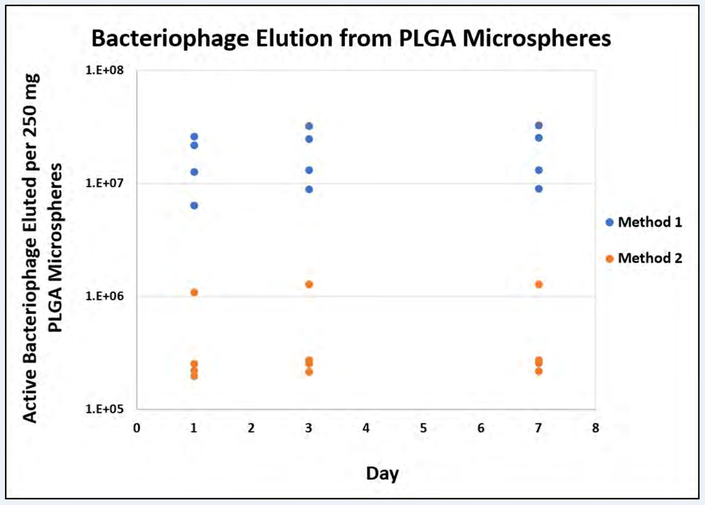

Figure 5. Active bacteriophage eluted from 250 mg of PLGA microspheres, manufactured using methods 1 (blue circles) or 2 (orange circles), after incubation in 1 ml of PBS at 37°C for 1, 3, and 7 days.

Discussion

- We performed a side-by-side comparison of two methods to manufacture bacteriophage-containing PLGA microspheres to determine which method resulted in the greatest elution of active bacteriophage and entrapment efficiency.
- Method 1 eluted a statistically greater number of active phage and had a greater entrapment efficiency.
- Further studies should be performed to determine the effectiveness of phage-containing PLGA microspheres against *S. aureus* in *in vitro* biofilms and in an *in vivo* animal model.

Conclusions

This comparison study evaluated two methods to produce bacteriophage-containing PLGA microspheres to determine the best method to generate the greatest entrapment efficiency and total active bacteriophage delivery over a 7-day period to develop an effective alternative to antibiotic treatment for osteomyelitis patients. Method 1 resulted in greater elution and entrapment efficiency across all trials.

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Acknowledgements

We thank Dr. Danielle Garsin for use of the CSLM, Drs. Anthony Maresso and Austen Terwilliger, TAILΦR Labs, Baylor College of Medicine for suppling the phage, and the Dean's Office at McGovern Medical School for financial support.

Case Report: Chromosomally Integrated HHV-6B in a Solid Organ Transplant Patient

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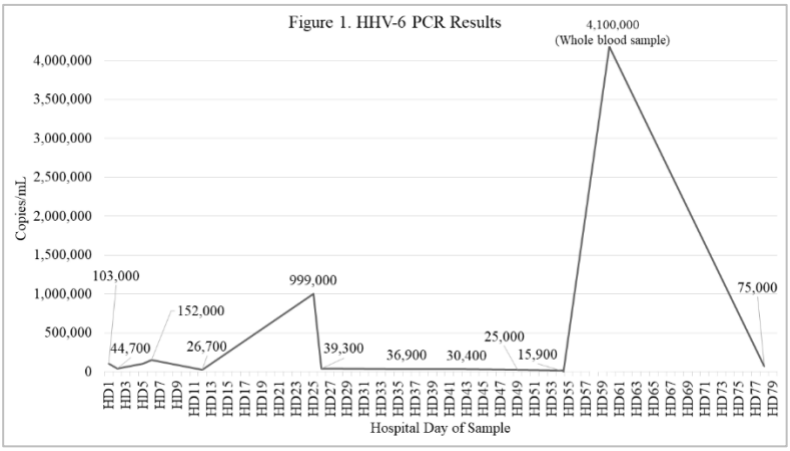
Background. Human herpesvirus-6A (HHV-6A) and -6B (HHV-6B), belong to the family *Herpesviridae*, a large group of ubiquitous DNA viruses with >90% seroprevalence in the adult population. After primary infection, HHV-6 will lay dormant in mononuclear cells. Chromosomally integrated HHV-6 (ciHHV-6) occurs in roughly 1% of the population worldwide. ciHHV-6 can be confirmed via testing for HHV-6 PCR in a nail clipping or hair follicle or by testing plasma in a digital droplet PCR (ddPCR) determining the ratio of HHV-6 to cellular DNA. Because of the sensitivity of contemporary PCR technology, several factors can confound or contribute to the diagnosis of ciHHV-6.

Hypothesis/Goals. We present a 34-year-old female solid organ transplant (SOT) recipient with laboratory-confirmed ciHHV-6B via ddPCR.

Methods. This was a single-patient case report and review of relevant literature.

Results. Seven months prior to our specified encounter, the patient underwent a heart and double lung transplant with basiliximab induction. One month before the specified encounter, she was diagnosed with HHV-6 encephalitis after polymerase chain reaction (PCR) detected HHV-6 from cerebrospinal fluid at 126,000 copies/mL. She was treated with 28 days of IV foscarnet and discharged. The specified encounter occurred one week later when she presented to the emergency room with a low grade fever (100.4º) and cough. The initial HHV-6 PCR sent on hospital day 1 (HD1) resulted at 103,000 copies/mL. The patient was maintained on daily foscarnet and later ganciclovir as HHV-6 PCRs were trended (Figure 1). On HD61, a whole blood HHV-6 PCR resulted with 4,100,000 copies/mL. On HD68, a plasma sample was sent for ddPCR resulting in a virus-to-cell ratio of 1.04, consistent with ciHHV-6B. After 76 days of foscarnet and 55 days of ganciclovir, antivirals were stopped, and the patient was discharged on HD79.



Conclusions. We present this case of ciHHV-6B diagnosed with ddPCR in a SOT patient to add to the growing body of literature characterizing this phenomenon. Trends in viral load, infectious symptoms, and the risks and benefits of treatment should be weighed when making treatment decisions for ciHHV-6 as viral PCRs alone will be confounded by the presence of viral DNA in the host's genome.

Acknowledgements. No external or internal funding was received for this project.

Case Report: Chromosomally Integrated HHV-6B in a Solid Organ Transplant Patient

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Introduction

- Human herpesvirus-6A (HHV-6A) and -6B (HHV-6B) are ubiquitous *Herpesviridae* and have >90% seroprevalence in the adult population[1]
- Reactivation of HHV-6B after primary infection can occur during periods of stress or systemic hypersensitivity reactions
- In immunocompromised patients, HHV-6B reactivation has been associated with encephalitis, colitis, hepatitis, pneumonitis, myocarditis, hypogammaglobinemia and corticosteroid use[2-8]
- In less than 1% of cases, the HHV-6 viral genome can integrate into the telomeres of host chromosomes, leading to chromosomally integrated HHV-6 (ciHHV-6)
- Given the sensitivity of contemporary polymerase chain reaction (PCR) testing, several factors can confound or contribute to the diagnosis of HHV-6 reactivation versus ciHHV-6

Case

We present a 34-year-old female patient with a past medical history of Lemierre's syndrome (treated with 21 days of meropenem), and Eisenmenger's syndrome requiring 6 sternotomies, right ventricle repair, pulmonary artery patch repair, and mechanical aortic valve replacement, ultimately leading to solid organ transplant

- Seven months prior to the specified encounter she underwent a heart and double lung transplant from a high-risk donor with basiliximab induction (CMV D-/R+, EBV D+/R+)

One month before the specified encounter she was admitted for acute hypoxemic respiratory failure and headache

- Lumbar puncture revealed HHV-6 126,000 copies/mL in the CSF and she was diagnosed with HHV-6 encephalitis
- She received 28 days of foscarnet and was discharged one week before the specified encounter

Case

The specified encounter occurred when the patient presented to the ED with fever, cough, and oxygen saturation of 77% on room air

- Initial HHV-6 PCR returned 103,000 copies/mL, and foscarnet was resumed
- She also underwent treatment for antibody-mediated rejection (C4d 20%)
- Repeat HHV-6 PCRs were trended, and ganciclovir was added (Figure 1)
- On Hospital Day 61 a whole blood HHV-6 PCR resulted with 4,100,000 copies/mL and ciHHV-6 entered the differential
    - A digital droplet PCR (ddPCR) was sent to the University of Washington and results were consistent with chromosomal integration (Figure 2)
- After confirmation of ciHHV-6B, all antivirals were stopped and the patient was able to be discharged 3 days later

Figure 1. HHV-6 PCR Results

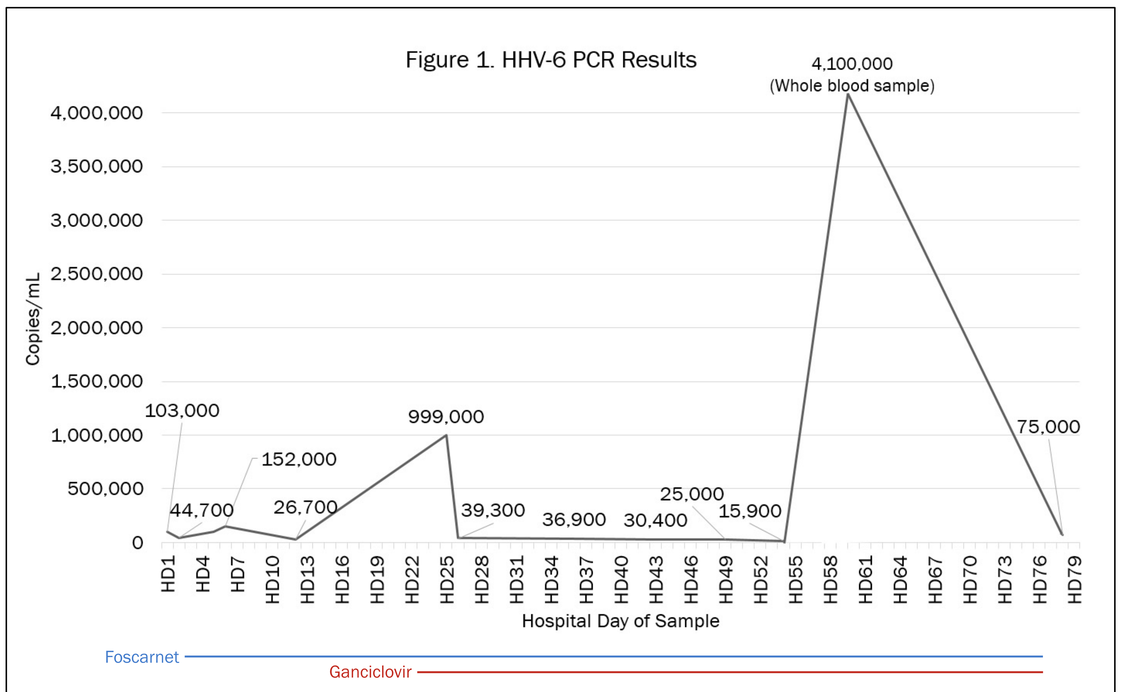

Figure 2. Digital Droplet PCR (ddPCR) Results

Comment: HHV6 CHROMOSOMAL INTERGRATION AND TYPING
Source: Plasma

Results Summary
HHV6 Integration and Typing
HHV6 Type A: None detected
HHV6 Type B: *Positive
HHV6 Virus to Cell Ratio: 1.04
HHV6 Chromo Intergrat Interp:
The HHV6 virus to cell ratio is approximately 1. Results are consistent with HHV6 chromosomal integration of the virus into the patient cell genome.

Discussion

- ciHHV-6 is exceedingly rare and is typically an inherited condition, though transmission via hematopoietic stem cell transplant has been documented[9]
- It is possible the sentinel event of HHV-6 encephalitis was a reactivation of ciHHV-6 as this patient had several characteristics associated with HHV-6 reactivation
- Individuals with ciHHV-6 can have elevated plasma PCR quantifications but it is more common to see even higher levels in whole blood samples; a whole blood quantification of >500,000 copies/mL generally indicates ciHHV-6[1]
- Diagnosis of ciHHV-6 can be confirmed by PCR quantification of hair or nail samples or via ddPCR indicating the ratio of host DNA to viral DNA is ~1

Conclusion

This case had certain characteristics associated both with HHV-6 reactivation and ciHHV-6, confounding the diagnosis of an already rare condition. Trends in viral load, infectious symptoms, and the risks and benefits of treatment should all be weighed when making treatment decisions for ciHHV-6.

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Intestinal Microbiome Composition and Carriage of Multidrug-resistant Organisms among ICU Patients in Colombian hospitals

Castro BE[1], Pacheco-Montealegre M[1], Parada A[1], Carvajal LP[1], Espitia C[1], Martinez J[1,2], Rincon S[1], Ordoñez K[3], Salcedo S[4], Guevara FG[2], Arias CA[5,6], Diaz L[1], Reyes J[1]

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Background. The gut microbiome represents a complex ecosystem with profound impact on the modulation of health and immunity. Alteration of the gut microbiota structure in critically-ill patients during ICU stay is likely to be a key factor associated with morbidity and mortality. Multidrug-resistant Organisms (MDRO) can dominate the gut microbiome with loss of commensal microbiota species. Our goal was to investigate the gut microbiome signatures, MDRO carriage among ICU patients in three Colombian hospitals.

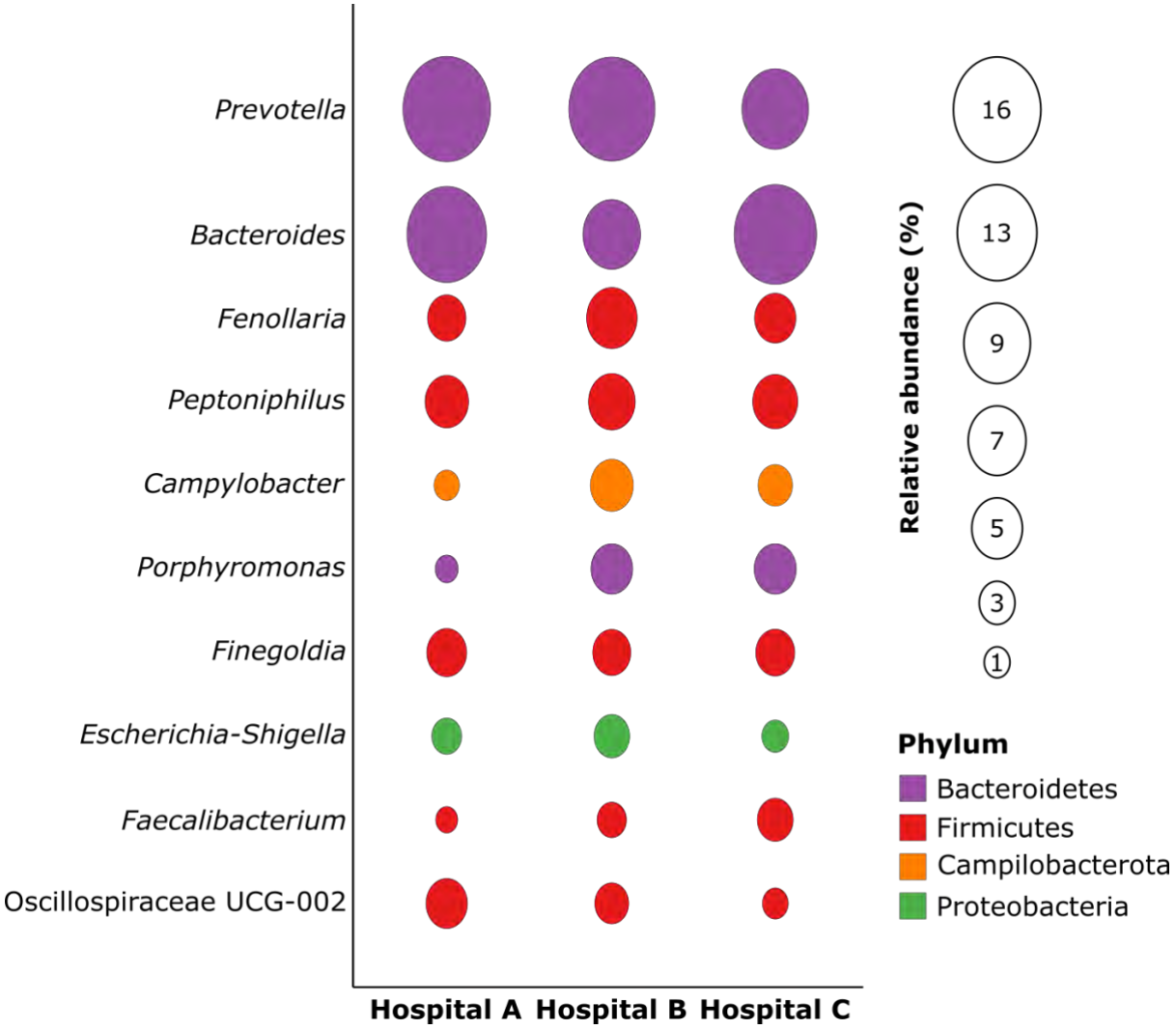
Methods: 131 critically-ill patients (>18 years old) from three cities in Colombia were prospectively included. Rectal swabs were collected upon admission at ICU and, subsequently, in a follow-up sample. The presence of carbapenem-resistant-Enterobacterales (CRE) and vancomycin-resistant-enterococci (VRE) was assessed by chromogenic-culture based screening. Microbiome analysis was investigated by 16S rRNA sequencing and amplicon sequence variant (ASV). Richness, Simpson, and Shannon indexes were calculated. Welch T's T-test was used to identify possible differences in specific taxa across the groups

Results: A total of 186 rectal swabs were obtained from 131 patients. Most of patients were men (64%); median age was 58 years. 64% of patients were admitted from home and 7% were considered hospital transfers. Charlson Comorbidity Index and Pitt Bacteremia Score were 6 (SD=2) and 4 (SD=3), respectively. VRE and CRE colonizers were identified in 30% and 12% of the patients, respectively. Co-colonization by CRE and VRE was observed in 5 patients. The most abundant taxa across the samples are shown in Fig.1. No significant differences of alpha diversity were noted between VRE or CRE-positive vs negative samples ($P>0.05$). However, low prevalence of *Mogibacterium, Enterococcus* and *Escherichia-Shigella* ($P<0.01$) was noted in VRE-positive samples. Whereas, low frequency of *Fenollaria, Fusobacterium* and *Ezakiella* ($P<0.01$) was observed in CRE-positive samples. Finally, comparing the microbiome upon admission of patients, we observed that patients who developed an infection (n=16) had less abundance of *Enterobacteriaceae, Succinivibrionaceae* and *Ruminococcus* ($P<0.01$).

Conclusions. Particular profiles of microbiome structure were observed among ICU patients colonized with MDRO pathogens from hospitals in Colombia. Microbiome characterization is likely to become a routine component and might provide biomarkers not only to treat infections but also to predict patient outcomes, which is particularly important in severe ill patients at ICUs.

Acknowledgements: This study was funded by Minciencias (COD 130880164152)

Figure 1. Microbiome composition of rectal swab samples from ICU patients. Bubble plot of the composition of most abundant taxa across 3 hospitals is shown. The color of the circles represents the Phylum of plotted taxa and bubble diameter show the relative abundance (%) of each taxa in each hospital.



Intestinal Microbiome Composition and Carriage of Multidrug-Resistant Organisms among ICU Patients in Colombian Hospitals

Castro BE[1], Pacheco-Montealegre M[1], Parada A[1], Carvajal LP[1], Espitia C[1], Martinez J[1,2], Rincon S[1], Ordoñez K[3], Slacedo S[4], Guevara F[2], Arias CA[5,6], Diaz L[1], Reyes J[1]

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Poster #18

ABSTRACT

Background: The gut microbiome represents a complex ecosystem with profound impact on the modulation of health and immunity. Alteration of the gut microbiota structure in critically-ill patients during ICU stay is likely to be a key factor associated with morbidity and mortality. Multidrug-resistant Organisms (MDRO) can dominate the gut microbiome with loss of commensal microbiota species. Our goal was to investigate the gut microbiome signatures, MDRO carriage among ICU patients in three Colombian hospitals.

Methods: 131 critically-ill patients (>18 years old) from three cities in Colombia were prospectively included. Rectal swabs were collected upon admission at ICU and, subsequently, in a follow-up sample. The presence of carbapenem-resistant-Enterobacterales (CRE) and vancomycin-resistant-enterococci (VRE) was assessed by chromogenic-culture based screening. Microbiome analysis was investigated by 16S rRNA sequencing and amplicon sequence variant (ASV). Richness, Simpson, and Shannon indexes were calculated. Welch T's T-test was used to identify possible differences in specific taxa across the groups

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Conclusions: Particular profiles of microbiome structure were observed among ICU patients colonized with MDRO pathogens from hospitals in Colombia. Microbiome characterization is likely to become a routine component and might provide biomarkers not only to treat infections but also to predict patient outcomes, which is particularly important in severe ill patients at ICUs.

BACKGROUND

Gram-negative bacilli and Gram positive cocci are an important cause of hospital infections and catalogued as a public health problem. They are classified by CDC as serious like Carbapenem-resistant Enterobacterales (CRE) and Acinetobacter and urgent threat pathogens as vancomycin-resistant Enterococci (VRE) and multidrug-resistant Pseudomonas[1-2]. Mainly, their ability to persisting in clinical environments is associated with resistance to antiseptics and antibiotics[3-4]. Patients in hospitals environments such as the ICU are at high risk of being colonized and infected[4,5]. The gut microbiome represents a complex ecosystem with profound impact on the modulation of health and immunity. Alteration of the gut microbiota structure in critically-ill patients during ICU stay is likely to be a key factor associated with morbidity and mortality. Multidrug-resistant Organisms (MDRO) can dominate the gut microbiome with loss of commensal microbiota species.

AIM

To investigate the gut microbiome signatures, MDRO carriage among ICU patients in three Colombian hospitals.

METHODS

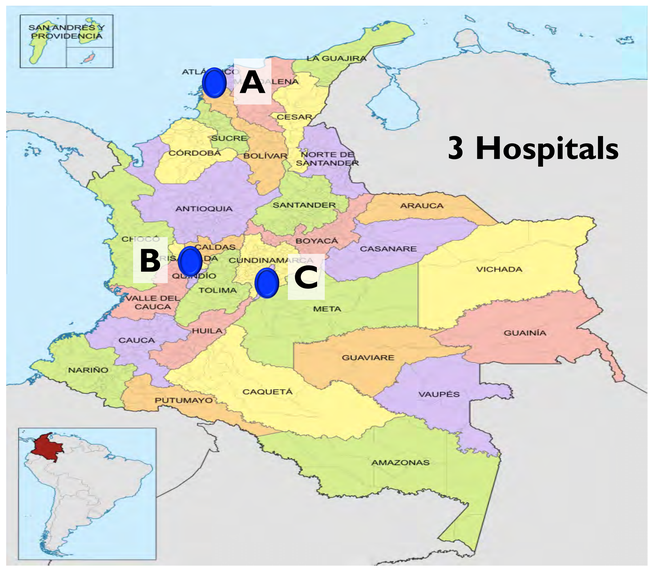


Figure 1. Location of participating hospitals

I
- Rectal swabs (1st) on admission at ICU.
- A follow-up sample (2nd)
- Screening in CHROMagar for CRE and VRE

II
- 16S rRNA Sequencing and Amplicon sequence variant (ASV)
- Calculation of Richness, Simpson, and Shannon indexes
- Differences analysis by Welch's T-test

Figure 2. Methodology used in this study.

RESULTS

I. Overall Sampling Data

A.

Hospital	Samples n (%)	Patients n (%)
Hospital A	13 (7)	8 (6)
Hospital B	131 (70)	93 (71)
Hospital C	42 (23)	30 (23)
TOTAL	186 (100)	131 (100)

B.

Sample day	Total samples	CRE-Col	VRE-Col
Zero	126 (68)	9 (56)	26 (66)
Follow-up	60 (32)	7 (44)	13 (34)
TOTAL	186 (100)	16 (100)	39 (100)

Table 1.A. Patients and Microbiome analyzed samples. **B.** MDRO colonization by samples.

II. Intestinal Colonization by MDRO at ICU patients

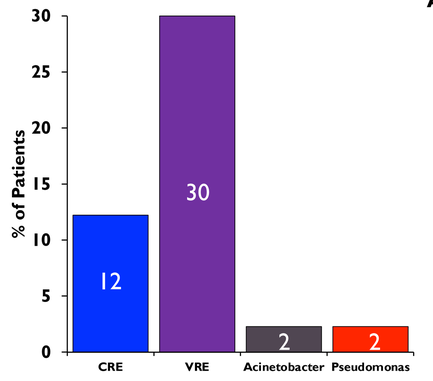
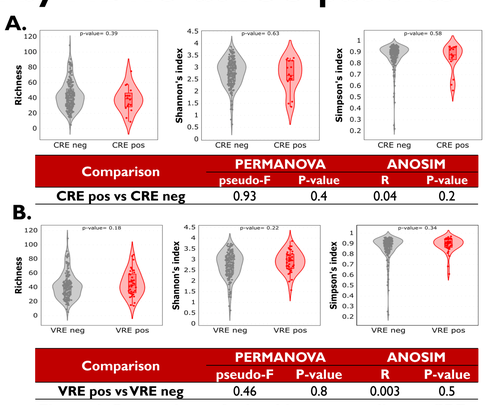


Figure 3. Distribution of MDRO in rectal swabs



Comparison	PERMANOVA pseudo-F	PERMANOVA P-value	ANOSIM R	ANOSIM P-value
CRE pos vs CRE neg	0.93	0.4	0.04	0.2
VRE pos vs VRE neg	0.46	0.8	0.003	0.5

Figure 4. Comparison of microbiome according to CRE (A) and VRE (B) presence in rectal swabs.

III. Microbiome Analysis at ICU patients

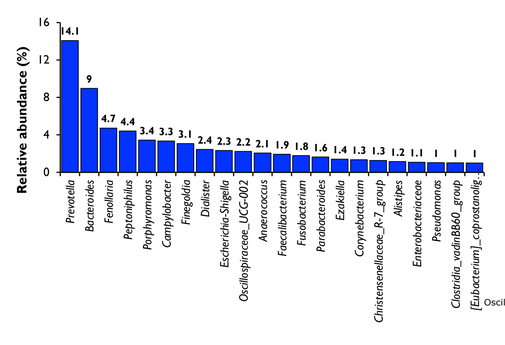


Figure 6. Relative abundance of rectal swabs

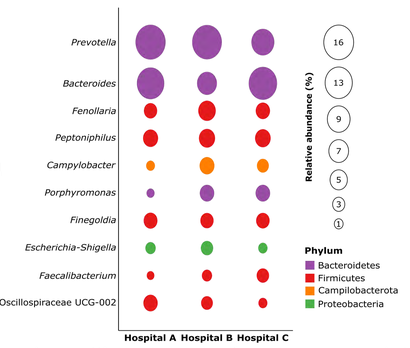
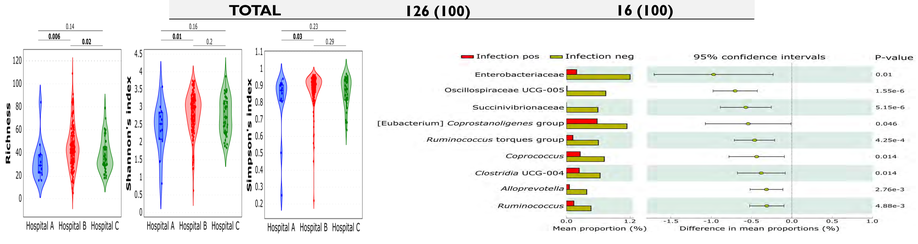


Figure 5. Microbial diversity of rectal swabs by hospital

IV. Analysis of microbiome (Zero-day) from patients who developed infection by MDRO in Colombian hospitals

Hospital	Total Patients N (%)	Infected Patients N (%)
Hospital A	8 (6)	2 (12)
Hospital B	90 (71)	9 (56)
Hospital C	28 (23)	5 (32)
TOTAL	126 (100)	16 (100)



CONCLUSIONS

- ✓ VRE and CRE colonizers were identified in 30% and 12% of the patients at Colombian ICU.
- ✓ We found not significant difference between the microbiota (alpha diversity) in patients colonized by CRE and VRE.
- ✓ The most abundant taxa across the samples are Prevotella and Bacteroides by sample and hospital.
- ✓ There are not significant differences between the zero-day samples of patients who developed infection and those who did not.

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NDM-5 Metallo-beta-lactamase in Patient Traveling from India: Superposition Aztreonam and Ceftazidime-Avibactam *in vitro* Testing and Molecular Sequencing

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Background
New Delhi metallo-β-lactamase (NDM) producing organisms pose significant challenges for healthcare providers due to limited therapeutic options. The combination of aztreonam and avibactam has been explored for the treatment of NDM-producing organisms.

Hypothesis/Goals
We report the isolation of an NDM-5-producing *Klebsiella pneumoniae* in a complex patient traveling from India. Additionally, we describe an *in vitro* susceptibility testing approach of aztreonam and ceftazidime-avibactam using a superposition method paired with confirmatory testing and molecular sequencing of resistance genes.

Methods
A modified Epsilometer test (Etest) strip superposition method was used to test the *in vitro* activity of aztreonam and ceftazidime-avibactam against the NDM-producing *K. pneumoniae*. The isolate was also sent for whole genome sequencing and tested for *in vitro* activity of aztreonam or ceftazidime in combination with avibactam using the broth microdilution checkerboard method.

Results
All zones of inhibition from the Etest superposition method were measured to be 22 mm in diameter (Clinical and Laboratory Standards Institute [CLSI] zone of inhibition for aztreonam susceptibility is ≥ 21 mm for Enterobacterales). The results of whole genome sequencing showed the *K. pneumoniae* isolate carried a metallo-β-lactamase (blaNDM-5), ESBL (blaCTX-M-15), and other β-lactamases (blaSHV-11, blaTEM-1). Through the broth microdilution checkerboard method, the isolate was found to be inhibited by aztreonam-avibactam at a MIC of 0.25 mg/L but was resistant to ceftazidime-avibactam (MIC >16 mg/L) and aztreonam alone (MIC >16 mg/L) according to CLSI breakpoints.

Conclusions
Our patient case adds to the growing evidence that supports combining aztreonam with ceftazidime-avibactam for *in vitro* activity against NDM-producing gram-negative bacteria. We illustrated the *in vitro* activity of this antibiotic combination via a modified Etest superposition method along with molecular characterization of resistance mechanisms and antibiotic synergy testing by broth microdilution.

Acknowledgements
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NDM-5 Metallo-beta-lactamase in Patient Traveling from India: Superposition Aztreonam and Ceftazidime-Avibactam *in vitro* Testing and Molecular Sequencing

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BACKGROUND

- Carbapenemase-producing organisms continue to spread globally at a remarkable rate. Of the carbapenemases, New Delhi metallo-β-lactamases (NDMs) specifically pose a challenge for providers. NDM is an Ambler class B metallo-β-lactamase (MBL) able to hydrolyze most β-lactams, including carbapenems, but not monobactams.
- Aztreonam (ATM) is stable against MBLs alone due to poor, nonproductive binding with the enzyme. However, it has limited clinical utility as organisms harboring NDMs typically coproduce ESBLs and/or AmpC enzymes that rapidly hydrolyze ATM. Avibactam (AVI), currently available only in the combination product ceftazidime/avibactam (CAZ/AVI), is a non-β-lactam β-lactamase inhibitor that expresses potent inhibition of most serine β-lactamases (e.g., KPC, CTX-M, TEM, and SHV), as well as class C (AmpC) and some class D (e.g., OXA-48) enzymes. Combining ATM with an advanced β-lactamase inhibitor like AVI provides the necessary protection from ESBLs and other broad-spectrum β-lactamases.
- The limited pharmacotherapeutic options and availability of specialized antimicrobial susceptibility testing present numerous conundrums for infectious disease specialists. Furthermore, timely in vitro testing of desired susceptibilities may be difficult to obtain depending on the laboratory.
- We report the isolation of an NDM-5-producing *Klebsiella pneumoniae* in a complex patient traveling from India and describe an *in vitro* susceptibility testing approach of ATM and CAZ/AVI using a superposition method paired with confirmatory testing and molecular sequencing of resistance genes.

CASE PRESENTATION

A 55-year-old female was transferred from a hospital in Coimbatore, India to our hospital in New Mexico. The patient was a resident of New Mexico who traveled to India 10 months prior to transfer for meditative reasons and holistic therapy, including acupuncture for joint pain. After transfer to our institution, a full body computerized tomography scan revealed multi-compartmental intramuscular abscesses in the right gluteal region, anterior and medial compartments of the right thigh, right iliopsoas muscle, and medial compartment of the left thigh. There were also abscesses noted in the right axilla which were drained, and cultures grew an NDM β-lactamase-producing *K. pneumoniae* susceptible only to tetracycline and minocycline (Xpert® Carba-R [Cepheid®], PhoenixTM NMIC 306 panel [BD Diagnostics, USA]). Of note, the isolate was resistant to CAZ/AVI with an MIC of greater than 8/4 mg/L.

METHODS

The *in vitro* testing of ATM and CAZ/AVI utilizing the Epsilometer test (Etest) strip superposition method has been described by Emeraud et al. We used a similar method; however, we modified it by substituting ATM 30 mcg Kirby Bauer (KB) disks for the ATM Etest strips used in the original method due to lack of availability of ATM Etests in the laboratory (Figure 1).

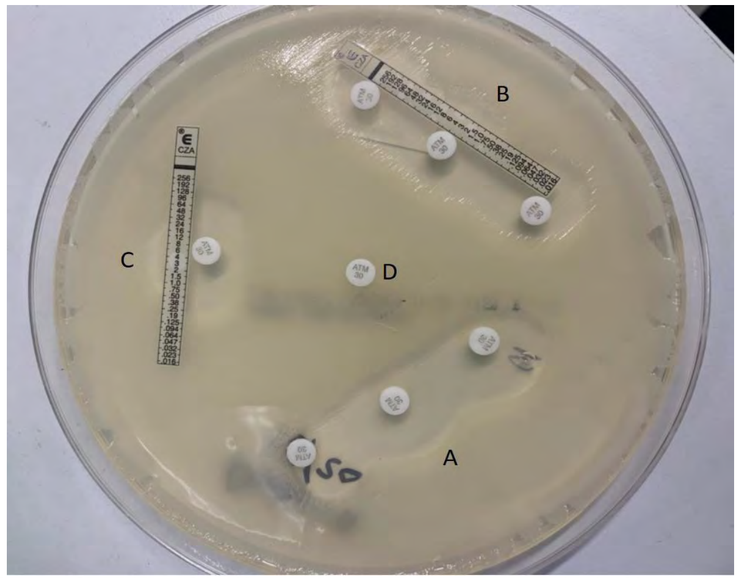
The isolate was sent to JMI Laboratories for whole genome sequencing and *in vitro* activity testing of ATM or CAZ in combination with AVI using a broth microdilution checkerboard method.

METHODS

- Mueller Hinton media were inoculated with a suspension of the NDM-5-producing *K. pneumoniae* isolate grown to a standard optic density of 0.5 McFarland units.
- Three Etest strips of CAZ/AVI (BioMerieux, USA) were placed on the lawn of inoculated bacteria (Figure 1a, 1b, and 1c).
- One of the CAZ/AVI Etest strips was set to dwell on the media for ten minutes, allowing the antibiotic to saturate the area. It was then removed, and three ATM KB disks were placed directly over the Etest imprint (Figure 1a).
- ATM KB disks were oriented next to the other two CAZ/AVI Etests at three different concentrations and at the center of the Etest strip (Figure 1b and 1c respectively).
- Multiple ATM KB disks were positioned along the CAZ/AVI Etest strip to assess microbial growth inhibition at varying CAZ/AVI concentrations.
- A single ATM KB disk was placed in the center of the plate to test for resistance to ATM alone (Figure 1d). A quality control procedure was simultaneously performed using *Escherichia coli* ATCC25922 isolate. The plates were incubated at 35ºC, and the antimicrobial zones of inhibition were measured twenty-four hours after the inoculation.

RESULTS

Figure 1: ATM and CAZ/AVI in vitro testing with modified superposition method



- **All zones of inhibition of the *K. pneumoniae* isolate were measured to be 22 mm in diameter (CLSI zone of inhibition for ATM susceptibility is ≥ 21 mm for Enterobacterales)**, including the zone of inhibition from where the CAZ/AVI Etest was placed for ten minutes and removed. This particular inhibitory zone is noted in Figure 1a.
- The control *E. coli* isolate was susceptible to all antimicrobial tests (results not shown).

RESULTS

The results of whole genome sequencing demonstrated the *K. pneumoniae* isolate carried an MBL (*bla*NDM-5), ESBL (*bla*CTX-M-15), and other β-lactamases (*bla*SHV-11, *bla*TEM-1). Porin mutations were identified in OmpK35, OmpK36, and OmpK37. The disruption in OmpK36 (G134_D135insDG, A183_T184insLSP, N227_L228insK, etc.) in particular is known to be associated with reduced susceptibility or resistance to β-lactams and carbapenems. In addition, alternations were also observed in penicillin binding proteins (PBP1, PBP2, PBP3).

Gene Group	Gene	Sequencing results
β-lactamases		bla_{NDM-5}, $bla_{CTX-M-15}$, bla_{SHV-11}, bla_{TEM-1}
Penicillin biding proteins	PBP1a	Alterations detected (D370N,T639A)
	PBP1b	Alterations detected (A407V,Q819P,Q820P,Q821P,Q829_Q829del)
	PBP2	Alterations detected (T85A,S90Q,N112T,N171S,D179N,H189R,D297E,L512Q)
	PBP3	Alterations detected (M6T,A33V,V41I,L370I,Q374K,E434A,I447M,N455S,L577Q,A578G)
Outer membrane protein	OmpK35	Alterations detected (T74I,P93T,V183I,G211S,V241I)
	OmpK36	Disruption detected (G134_D135insDG,A183_T184insLSP,G189T,Q191L,V202L,F207Y,H218N,T222L,D223G, N227S,N227_L228insK,V229A,N232R,L311I,R345H,V349I)
	OmpK37	Alterations detected (V19A,S88T,N230G,M233Q,T234H,Q235Y,N237H,N237_A238insTERY,R239K,E244D,N274S,D275T, D275_G276insSSTNGG,V277I,V295G,D350G,S353K,K356E)

The *K. pneumoniae* isolate was tested for *in vitro* activity of ATM or CAZ in combination with AVI (fixed at 4mg/L) using the broth microdilution checkerboard method:

- **The isolate was found to be inhibited by ATM/AVI at a MIC of 0.25 mg/L but was resistant to CAZ/AVI (MIC >16 mg/L) and ATM alone (MIC >16 mg/L) according to CLSI breakpoints.**

The low MIC value of ATM/AVI indicated potent *in vitro* activity of the combination against the isolate that carried MBL, ESBL, and non-enzymatic resistance mechanisms. This result correlated with observations from the superposition method where the three ATM inhibition zone sizes were similar along the concentration gradients of CAZ/AVI Etest strips (Figure 1a and 1b), and no inhibition by CAZ/AVI except by the ATM disk (Figure 1c).

CONCLUSIONS

We illustrated the *in vitro* activity of ATM and CAZ/AVI combination via a modified Etest/KB disk superposition method coupled with molecular gene sequencing and broth microdilution checkerboard testing. Compared to more sophisticated susceptibility methods, Etests and KB disks are common in laboratories and require less specialized technology or training. Thus, a superposition method using Etests or Etests with KB disks serves as a simple and easily accessible technique for determining the MIC of ATM and AVI in combination. Larger studies are needed to confirm the most efficient and accurate method. The ability to rapidly identify these highly resistant bacteria and determine antibiotic susceptibility is crucial when pharmacotherapeutic options remain limited.

DISCLOSURES/ACKNOWLEDGEMENTS

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J.S. is a speaker's bureau member for AbbVie, and L.Y.L. is an employee of AbbVie. All other authors: none to declare
References available upon request

Emergence of Tn1549-mediated vanB vancomycin Resistance in Multidrug-resistant Enterococcus faecalis ST6 in the United States

Simar SR[1], Hanson BM[1], Tran TT[2,3], Rydell KB[2,3], Contreras GA[4], Axell-House, DB[2,3], Cifuentes R[5], Abbo L[5], Sahasrabhojane P[6], Shelburne SA[6], Dinh AQ[2,3], Panesso D[2,3], Munita JM[7], Arias CA[2,3]

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Background: Enterococci are a significant cause of hospital-associated infections and are becoming increasingly resistant to available treatments. However, though *Enterococcus faecalis* (Efs) remain the dominant species causing bloodstream infections, reports of vancomycin-resistant *E. faecalis* (VREfs) in the United States are exceedingly rare. Here, we characterize the genomic context of three VREfs isolates with *vanB*-mediated vancomycin resistance in three patients in U.S. hospitals in order to provide insight into a rarely characterized mechanism of vancomycin resistance in this species.

Methods: VREfs isolates were collected through the Vancomycin-Resistant Enterococci Outcomes (VENOUS) study, a prospective study of enterococcal bloodstream infection outcomes in a global cohort. Short- and long-read genomic sequencing were performed with Illumina NextSeq 2000 and Oxford Nanopore Technologies GridION platforms, respectively. An in-house bioinformatics pipeline was used for hybrid genome assembly.

Results: Of the 203 Efs in the VENOUS cohort, VREfs isolates harboring *vanB* from three unique patients (1.4%) were identified—two in patients from two hospital systems in Houston, TX (2018, 2020), and one from a hospital system in Miami, FL (2020). All isolates were ST6, had chromosomally located *vanB2* operons, and were phenotypically non-susceptible to vancomycin with MICs all ≥32ug/mL. Interestingly, the two Houston, TX isolates possessed nearly identical Tn*1549* conjugative transposons flanked by IS*256* elements, which has not been previously characterized. These isolates differed by 181 SNPs and possessed similar but unique plasmid repertoires but had no identified epidemiological link. A complete Tn*1549* transposon was also identified in the Miami, FL VREfs but was not flanked by IS elements, suggesting a different mechanism of mobilization. Interestingly, the Miami, FL VREfs had a roughly 2.8Mb chromosomal inversion in tRNA regions relative to the two Houston, TX isolates. In addition to *vanB*, all isolates possessed AMR genes conferring erythromycin, high-level aminoglycoside, and tetracycline resistance.

Conclusions: Here, we have identified three spatiotemporally distinct occurrences of Tn*1549*-mediated *vanB* vancomycin resistance acquisition in Efs in the U.S. To the best of our knowledge, this is a novel and emergent genotype in Efs not previously characterized in the U.S. Our results highlight a need for constant surveillance of emergent infectious diseases threats.

Funding sources

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Emergence of Tn*1549*-mediated *vanB* vancomycin resistance in multidrug-resistant *Enterococcus faecalis* ST6 in the United States

Simar SR[1], Hanson BM[1], Tran TT[2,3], Rydell KB[2,3], Contreras GA[4], Axell-House DB[2,3], Cifuentes R[5], Abbo L[5], Sahasrabhojane P[6], Shelburne SA[6], Dinh AQ[2,3], Panesso D[2,3], Munita JM[7], Arias CA[2,3]

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INTRODUCTION/BACKGROUND

- Vancomycin-resistant enterococci (VRE) are a leading cause of hospital-acquired bloodstream infections and a pathogen of increasing importance.
- Reports of vancomycin-resistant *E. faecalis* remain rare in the U.S.
- Prospective surveillance studies enable the early detection of uncommon antimicrobial resistance mechanisms and can highlight emerging AMR trends of concern.
- Here, we characterize the genomic context of three vancomycin-resistant *E. faecalis* (VREfs) isolates with *vanB*-mediated vancomycin resistance in three patients hospitalized in Houston, TX, and Miami, FL, in order to provide insight into a rarely characterized mechanism of vancomycin resistance in this species.

METHODS

Isolate and clinical data collection: Isolates were gathered as part of the Vancomycin-Resistant Enterococci Outcomes Study (VENOUS), a prospective, observational study conducted in 13 hospital systems globally. The isolates were identified as causal agents of bloodstream infections and were obtained through each institution's clinical microbiology laboratory. Clinical data was abstracted from each institution's electronic medical record system and managed in REDCap (2) hosted at UTHealth and MD Anderson.

Sample processing and whole-genome sequencing: Short-read sequencing was performed with the Illumina NextSeq 2000 with 2x150 paired-end reads. The data was assembled and quality-checked using an in-house script. Long-read sequencing was performed using the Oxford Nanopore Rapid Barcoding Kit (SQK-RBK004) on the GridION X5 (Oxford, UK). Hybrid assemblies using both short- and long-read sequencing output were created using a custom pipeline (https://github.com/wshropshire/flye_hybrid_assembly_pipeline).

Data analysis and visualization: Multi-locus sequence typing was performed with mlst (mlst (https://github.com/tseemann/mlst). Resistance genes were identified using ABRicate (https://github.com/tseemann/abricate) utilizing the Comprehensive Antimicrobial Resistance Database (3). Assemblies were annotated with Prokka v1.14.5. Single nucleotide polymorphisms (SNPs) were called using snippy v4.6.0. Genomes were visualized using SnapGene v5.0.8. Genomic comparison figures were created with EasyFig v2.2.2.

Conjugation assays: A filter mating protocol for conjugative transfer was used to test the mobilizability of Tn*1549*. E. faecalis OG1RF was used as a donor strain, and a positive control donor-recipient pair of *E. faecium* TX1330RF and ERV99 (4), respectively, were included to validate assay performance. Vancomycin and fusidic acid concentrations of 8ug/mL and 25ug/mL, respectively, were used for transconjugant selection for each donor-recipient pair, including the positive control. Assays were performed with donor-recipient ratios of both 1:10 and 10:1.

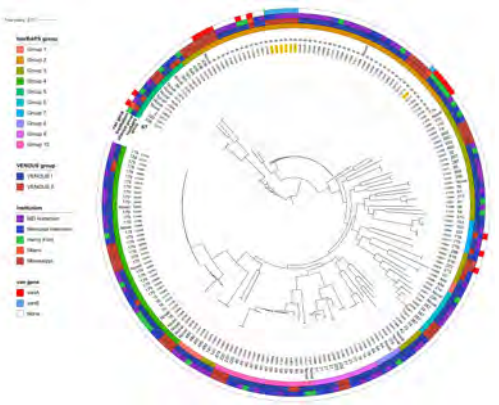


Figure 1. Midpoint-rooted, core genome-aligned maximum likelihood phylogenetic tree of VENOUS *E. faecalis* isolates. Groups of statistically-determined relatedness (hierBAPS groups), VENOUS I/II collection phase, institution of origin, and *van* gene presence/absence and type are represented as colored bars. Highlighted names indicate *vanB*-containing Efs explored in this analysis.

Patient and isolate characterization. Patients with *vanB* vancomycin-resistant *E. faecalis* were found in three geographically distinct hospitals, though two originated from the same city (**Figure 1**). The two Houston, TX, isolates were recovered two years apart. Two of the three patients had a history of hospitalization in the past year, and one patient had no recorded history of antibiotic use in the 30 days prior to admission (**Table 1**). The isolate obtained from each patient's index blood culture (**Table 2**, red text) was used for subsequent analyses. In both cases of persistent bacteremia (>1 positive blood culture), the *vanB* gene was maintained throughout the course of infection.

RESULTS

Table 1. Patient demographics and hospital stay information.

	Patient 1	Patient 2	Patient 3
Institution	Houston, TX CC	Miami, FL HS	Houston, TX HS
Year of admission	2018	2020	2020
Age, years	40	79	51
Length of stay, days	29	83	15
Location prior to admission	Community/home	LTAC	Community/home
Location upon admission	Non-ICU	ICU	ICU
Antibiotic history 30 days prior to admission	FEP, LZD, MIN	FEP, MEM, VAN	None
History of hospitalization in past year?	Yes	No	Yes
No. of positive blood cultures during hospitalization	3	1	5
Antibiotics given during hospitalization	AMP, CRO, LZD, MEM	LZD	AMP, CRO, LZD, MEM

CC- cancer center; HS- hospital system; LTAC- long-term acute care; FEP- cefepime; LZD- linezolid; MIN- minocycline; SAM- ampicillin-sulbactam; DAP- daptomycin; MEM- meropenem; VAN- vancomycin; AMP- ampicillin; CRO- ceftriaxone

Table 2. Isolate characterization.

Patient	Isolate	Location	Timing of blood culture from day of admission (days)	ST	*van* gene identified
1	**C5742 (HTX1)**	Houston, TX CC	0	6	*vanB*
	C5743	Houston, TX CC	1	6	*vanB*
	C5745	Houston, TX CC	3	6	*vanB*
2	**C5758 (MFL1)**	Miami, FL HS	0	6	*vanB*
3	**C5844 (HTX2)**	Houston, TX HS	0	6	*vanB*
	C5846	Houston, TX HS	4	6	*vanB*

CC-cancer center; HS-hospital system; ST- sequence type
Red denotes index isolates that were included in this analysis.

Table 3. Resistance gene repertoire.

	aac(6')-ie-aph(2'')-ia	aph(3')-iiia	*ermB*	*sat4*	*aad(6)*	*dfrE*	*dfrF*	*efrA*	*efrB*	*emeA*	*lsaA*	*tetM*	*vanB*
HTX1	*	+	++	+	+	*	*	*	*	*	*	**	*
MFL1	*	+	+	+	+	*	*	*	*	*	*	**	*
HTX2	*		+			*	*	*	*	*	*	**	*

Copies of gene present in genome: 2 … 0

Location of gene(s)- chromosome: *
Location of gene(s)- plasmid: +
Shading and number of symbols correspond to the number of copies of each gene present in the genome.

Table 4. Whole-genome single-nucleotide polymorphism (wgSNP) matrix.

	HTX1	MFL1	HTX2
HTX1		727	180
MFL1	739		761
HTX2	181	755	

Isolate resistance gene repertoire and similarity. Isolates possessed similar AMR gene content, though the two Houston, TX, isolates both harbored two copies of erythromycin resistance gene *ermB* on plasmids (**Table 3**). Isolate HTX2 was missing 3 AMR genes found in other isolates– APH(3')-IIIa, SAT-4, and *aad(6)*. All isolates harbored two copies of *dfrF* and two copies of chromosomally-located *tetM* genes. Unsurprisingly, the two Houston, TX, isolates were more similar to each other than to MFL1, differing by 181 whole-genome SNPs (**Table 4**).

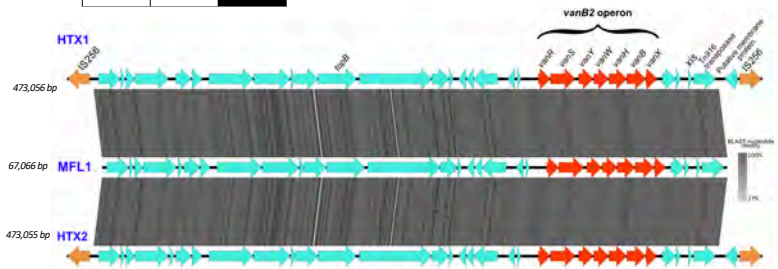


Figure 2. Characterization of transposon Tn*1549* carrying the *vanB2* operon. All three *vanB*-harboring *E. faecalis* isolates had chromosomally-located *vanB2* operons. Each isolate also possessed Tn*1549* conjugative transposons; interestingly, this transposon was inserted in identical locations in the two Houston, TX, isolates, and it was also flanked by IS*256* insertion sequences, which has not been characterized prior to this.

Figure 3. Depiction of chromosomal inversion site.

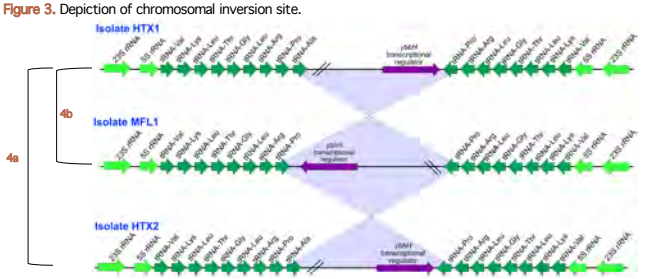


Figure 4a. Dot plot of HTX1 chromosome vs. HTX2 chromosome.

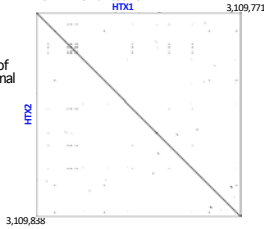


Figure 4b. Dot plot of HTX1 chromosome vs. MFL1 chromosome.

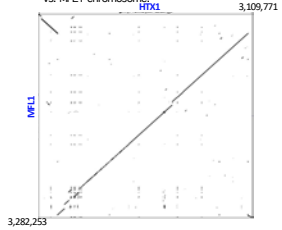
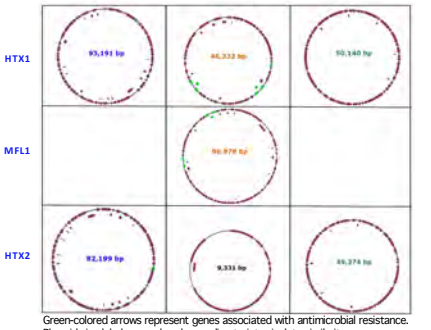


Figure 4. Depiction of chromosomal synteny.

Figures 3 and 4. Chromosomal inversion. Interestingly, a ~2.9MB chromosomal inversion was noted between isolates HTX1/HTX2 and MFL1. The inversion occurred within tRNA regions, which are known "hotspots" of genomic integration and recombination (**Figures 3, 4a, 4b**).
In MFL1, the *vanB* operon is not inside the area of inversion. While the driving force behind the chromosomal rearrangements in the Houston vs. Miami isolates is unknown, it is believed that acquisition of the *vanB* operon occurs independently of this chromosomal rearrangement.

Figure 5. Plasmid repertoire. Isolates HTX1 and HTX2 from Houston, TX, both harbored three plasmids, though only two of the three were similar between isolates. Isolate MFL1 harbored only one plasmid that was larger than but similar to a pAM373-like plasmid seen in Isolate HTX1.



Green-colored arrows represent genes associated with antimicrobial resistance.
Plasmid size labels are colored according to inter-isolate similarity.

Mobilizability of Tn*1549*. Conjugative transfer by filter mating was attempted for each of the three strains, using *E. faecalis* OG1RF as a donor. Under the chosen conditions, transfer was not achieved in any of the strains.

CONCLUSIONS

- Three spatiotemporally distinct occurrences of ST6 *E. faecalis* harboring *vanB* on Tn*1549* have been identified in the United States.
- To the best of our knowledge, this is a novel and emergent genotype in Efs not previously characterized in the U.S.
- The relatively conserved genomic context of the *vanB* operon in distinct genomic backgrounds indicates that this mechanism of resistance may be transmissible, highlighting a potential trend of emerging resistance in *E. faecalis*. However, efforts to induce mobilization of Tn*1549* in these strains has been unsuccessful thus far.

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Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES
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Characterization of the Antimicrobial Susceptibility Patterns and Biofilm Formation During Staphylococcal Medical Device Infection

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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. The most common complication following protheses placement is breast implant-associated infections (IAIs), which occur in 2%-29% of cases and can result in significant morbidity, including tissue necrosis and disfigurement. IAIs 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 IAIs, major efforts, including the administration of prophylactic antibiotic pocket irrigants, have been implemented, often with conflicting results. Importantly, the most common etiologic agents causing IAI are staphylococci, including *Staphylococcus aureus* (SA) and *Staphylococcus epidermidis* (SE). Studies investigating the host-pathogen-device interactions that promote SA and SE IAI are needed to develop more effective prevention or treatment strategies.

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

Methods. To define the mechanisms that promote staphylococcal IAI, 2 SA and 5 SE strains were isolated from women requiring explanation of breast implants due to infection. Minimum inhibitory concentration (MIC) assays were performed using antibiotics commonly used in pocket irrigants to determine the antimicrobial susceptibility patterns. Additionally, we assessed these strains for the ability to form biofilm in the presence of different host proteins, which coat breast implants post placement. We also assessed how host-protein interactions and biofilm formation affect the MIC needed for each strain. Lastly, a mouse model of IAI was developed to investigate the host-pathogen interactions that promote infection.

Results. These studies indicate that all SA strains were sensitive to gentamicin and cefazolin, but resistant to bacitracin. Additionally, most (4/5) SE strains were sensitive to gentamicin and cefazolin, but resistant to bacitracin, while only one was resistant to gentamicin and cefazolin, but sensitive to bacitracin. Furthermore, SA and SE biofilm formation was enhanced in the presence of human plasma and the host protein collagen. Lastly, the mouse model demonstrates that SE requires the breast implant to alter the environment to cause disease, while SA readily infects and does not require further alternations.

Conclusions. Together with our previous studies these data indicate that SA and SE interact with host proteins deposited on the implant surface, which may promote IAI. IAIs are a dreaded complication following device placement, as treatment options are limited. This study highlights specific host-staphylococcal interactions that may be targeted for the development of non-antibiotic therapies.

Acknowledgements. This work was supported by a grant from the Plastic Surgery Foundation.

Characterization of the Antimicrobial Susceptibility Patterns and Biofilm Formation During Staphylococcal Medical Device Infection

UTHealth The University of Texas Health Science Center at Houston | McGovern Medical School

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Background

- Breast implants have high infection rates
  - Up to 30%
- Treatment requires implant removal
- Prevention is a priority
  - Prophylactic antibiotic usage
- Staphylococci cause >50% of all breast implant infections
  - *S. aureus* (SA) and *S. epi* (SE)

Goals

Understand why prophylactic antibiotic use fails to reduce infection rates

1. Determine antibiotic susceptibility patterns of SA and SE breast implant infection isolates
2. Identify mechanisms that promote recalcitrance

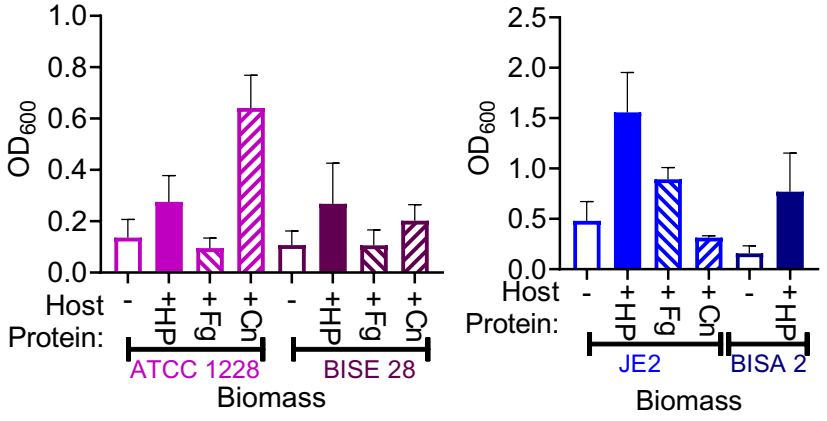
Results

Antimicrobial Susceptibility Patterns:

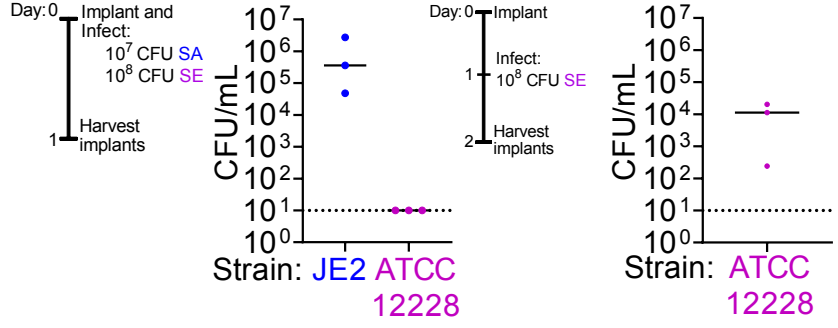
Strain	Infection (site)	Gent MIC	Cefa MIC	Bac MIC
ATCC12228	No (environment)	S	R	S
BIS28	No (breast implant)	S	S	I
BISE30	Yes (breast implant)	S	R	R
BISE31	Yes (breast implant)	S	R	R
BISE32	Yes (breast implant)	R	R	S
BISE33	Yes (breast implant)	S	R	R
BISE34	Yes (breast implant)	S	S	S
JE2	Yes (skin)	S	S	R
BISA1	Yes (breast implant)	S	S	R
BISA2	Yes (breast implant)	S	S	R

Gent=Gentamicin; Bac=Bacitracin; Cef=Cefazolin

Host Proteins Enhance Biofilm:



Mouse Model of Breast Implant Infection



Conclusions

All SA breast implant isolates were sensitive to Gent and Cefa, but resistant to Bac. All but one SE breast implant isolates were sensitive to Gent but resistant to Cefa, and two were resistant to Bac. Additionally, the presence of host proteins enhance SA and SE biofilm formation. Lastly, SA and SE infect breast implants in a mouse model.

Acknowledgements

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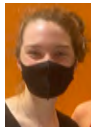
THE PLASTIC SURGERY FOUNDATION



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

The lipopeptide daptomycin (DAP) is an antibiotic frequently used a drug of last resort against Gram-positive Multi-Drug Resistant pathogens such as vancomycin-resistant enterococci. Unsurprisingly, as the use of DAP has increased, the number of pathogens evolving resistance has increased as well and threatens to limit the efficacy of this important drug. Inhibition of the major antibiotic stress pathway (LiaFSR) responsible for DAP resistance has been suggested as a strategy to retain the efficacy of DAP. Such a strategy was shown to be effective, however alternative pathways to resistance were identified including some with adaptive mutations in MurAA ($MurAA^{A149E}$). 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. Position 149 is distal to the MurAA active site and high-resolution structures of MurAA in complex with both inhibitor, fosfomycin, and substrate, UDP-N-acetylglucosamine along with steady-state substrate kinetics assays did not reveal substantial changes in either structure or activity of $MurAA^{A149E}$. However, cells expressing $MurAA^{A149E}$ had increased susceptibility to glycosyl hydrolases such as lysozyme consistent with changes in the cell wall. This is important as an adaptive pathway that increases susceptibility to glycosyl hydrolases would suggest increased *in vivo* sensitivity that would support the notion that inhibition of the LiaFSR pathway could be an excellent means to increasing DAP efficacy. Interestingly, the adaptive mutation in $MurAA^{A149E}$ increased affinity 16-fold for MurG. MurG performs the last intracellular step of peptidoglycan synthesis. In *Bacillus subtilis*, DAP has been shown to insert preferentially into the membrane at division septa leading to mislocalization of critical cell division proteins including MurG.

Hypothesis

MurAA and MurG localize at division septa and thus $MurAA^{A149E}$ may contribute to cellular fitness during DAP exposure by increasing the stability of MurAA-MurG interactions at the division septa to indirectly decrease DAP induced mislocalization.

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. MurAA localizes principally at septa and poles in DAP susceptible strain and A149E mutation can partially restore the MurAA-MurG mislocalization induced by DAP attack.

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

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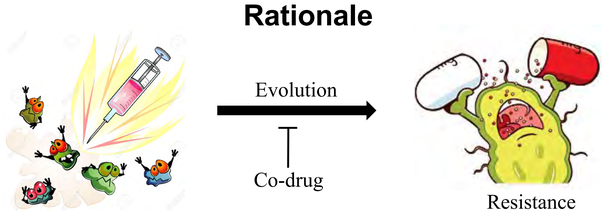
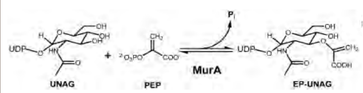
Abstract

Overuse of antibiotics has contributed to the evolution of multi-drug resistance and as a result, bacterial infections have re-emerged as a critical crisis in human health. Among these, enterococci infections pose a serious threat. Enterococci cause 66,000 infections in the United States each year and resistance to the frontline antibiotic, vancomycin, leaves limited treatment options for patients. The CDC estimates that vancomycin resistant enterococci (VRE) are responsible for about 1,300 deaths annually in the U.S. Daptomycin (DAP) is an antibiotic of last resort for many patients and is used frequently to treat VRE infections. Less than 2% of enterococci isolates are DAP nonsusceptible, indicating the high efficiency of DAP and thus, highlighting the importance of maintaining the efficacy of this drug in the absence of other viable treatment options. A clear understanding of the biochemical basis of resistance can provide targets and pathways for the development of new therapeutic approaches.

Rationale

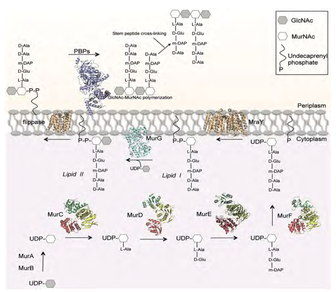


Previous studies from our lab using experimental evolution to identify adaptive strategies to DAP resistance in *Enterococcus faecium* revealed that mutations in murAA can provide an important alternative evolutionary trajectory to resistance when the LiaFSR stress response system is inhibited. MurAA 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.



Reactions catalyzed by MurA

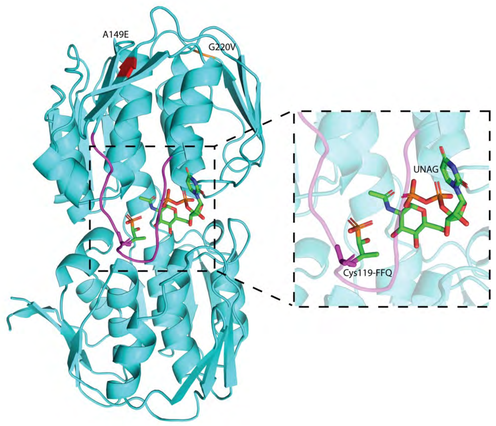
(Zhu et al., 2012)



Peptidoglycan Biosynthesis Pathway

(Laddomada et al., 2019)

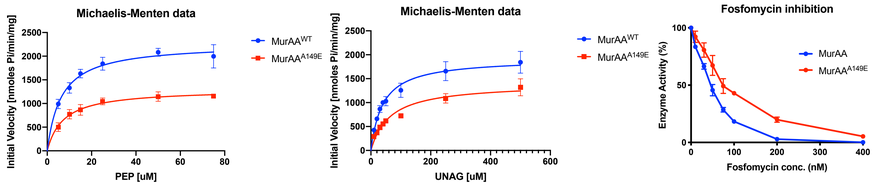
E.faecium MurAA with UNAG and fosfomycin



Two Domain Topology

- Active site Cys119 locates within the flexible loop (Ala114-Ile125)
- The active site loop adopts different conformations depending on the ligand binding.
- A149E mutation locates within one domain.

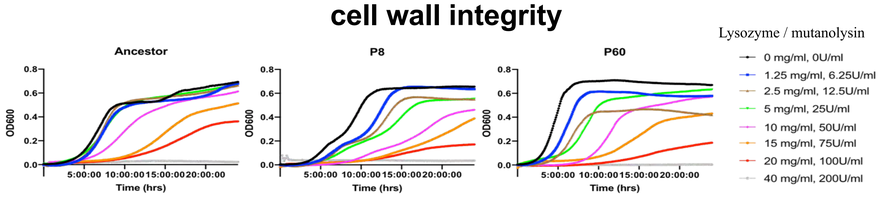
MurAA^{A149E} is Less Active than MurAA^{WT}



Kinetic parameters of MurAA^{WT} and MurAA^{A149E}

	Vmax for PEP [nmoles·min^{-1}·mg^{-1}]	Km for PEP [uM]	Vmax for UNAG [nmoles·min^{-1}·mg^{-1}]	Km for UNAG [uM]	IC_{50} for FFQ [nM]
MurAA^{WT}	2264.67 ± 77.27	6.353 ± 0.8807	1921.91 ± 68.40	39.81 ± 4.35	46.22 ± 2.85
MurAAA^{149E}	1302.17 ± 49.08	7.263 ± 1.048	1400.71 ± 66.11	63.18 ± 8.25	74.91 ± 10.16

Strains obtained MurAA^{A149E} have weaker cell wall integrity

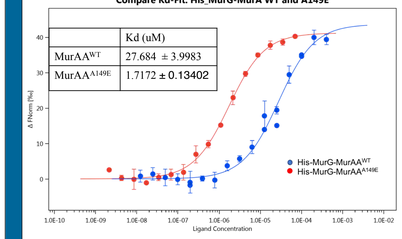


The above results indicate that strains obtained MurAA^{A149E} mutation are likely to have weaker cell wall integrity and take longer time to get used to the stress environment. The integrity of cell wall in *E.feacium* could demonstrate the effect of MurAA on cell wall.

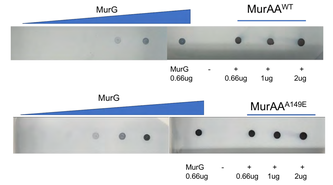
P8: MurAA^{A149E}, cls^{A20D}, caps^{A70E}, entfae_64^{Y83*}.
P60: MurAA^{A149E}, cls^{N13I}, entfae_126^{V30*}, repA plasmid 1(+214).

The binding affinity of MurG - MurAA^{A149E} is greater compared to that of MurG - MurAA^{WT}

Microscale Thermophoresis

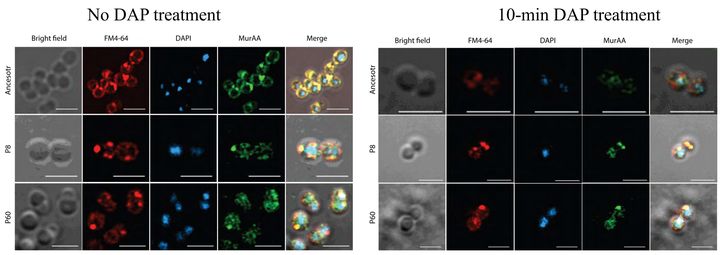


Dot Plot Assay showed a clear protein protein interaction of MurAA-MurG



MurA	MurG
First step of PG synthesis	Last step of PG synthesis
Enolpyruvate transferase	peripheral glycosyltransferase Catalyze the reaction forming lipid II

MurAA^{A149E} could partial restore the localization of MurG



Conclusions & Future Plan

- Septa localized MurAA-MurG provides sufficient supply of lipid II utilized by peptidoglycan synthesis
- In P8 and P60, the enzyme probably is not able to generate lipid II efficiently in the location utilized by the cells.
- MurAA A149E could retain partial colocalization with the damaged membrane.
- MurAA mutation doesn't seem to be optimal evolutionary strategies for a human pathogen because of the increased sensitivity to mutanolysin and lysozyme

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Poster Presentation Talks Day 2

First Name	Last Name	Institution	Poster Title	Day of presentation
Eva	Amenta	BCM	*Implementation Outcomes in an Antibiotic Stewardship Program (Kicking CAUTI) in Four Veterans Hospitals Correlated with Clinical Outcomes*	2
Advait	Balaji	RU	*SeqScreen: Accurate and Sensitive Functional Screening of Pathogenic Sequences via Ensemble Learning*	2
Ayan	Chatterjee	UTH	*The Role and Dynamics Of Ethanolamine-Utilizing Bacterial Microcompartments*	2
Sofia	Costa	Universidade NOVA de Lisboa	*Increased Virulence Potential Among Antimicrobial Resistant Coagulase-Positive Staphylococci Associated with Animal Pyoderma*	2
Taryn	Eubank	UH	*An Epidemiologic Exploration of Vancomycin Resistance in Clostridioides difficile*	2
Marc	Gohel	MDA	*Using Whole Genome Sequencing to Genetically Profile and Analyze Escherichia coli Isolates with Varying Resistance to β-lactam/β-lactamase Inhibitor Combinations*	2
Sonia	Gomez	Laboratorio Nacional de Referencia en Resistencia a los Antimicrobianos	*Carbapenemase-producing Extraintestinal Pathogenic Escherichia coli from Argentina: Clonal Diversity and Predominance of Hyperepidemic Clones CC10 and CC131*	2

Poster Presentation Talks Day 2

Holly	Hoffman	Paratekpharma	*Comparison of Healthcare Resource Utilization (HRU) Among Adult Patients Treated with Omadacycline (OMC) for Acute Bacterial Skin and Skin Structure Infections (ABSSSI) or Community-Acquired Bacterial Pneumonia (CABP) in the 30 Days Pre- and Post-OMC Prescription (Rx)*	2
Joseph	Hornak	UTMB	*Delafloxacin in Clinical Practice: A Single Center Study*	2
Iordanis	Kesisoglou	UH	*Seeing the Invisible: Deciphering Spectrophotometry-Based Time-Kill Measurements to Guide the Design of Antibiotic Dosing Regimens*	2
Paul	Nicholls	BCM	*Antibiotic Interactions with Bacteriophage in Pseudomonas Biofilms and Planktonic Environments*	2
Diana	Panesso	HMRI	*Role of the LiaF in the LiaR-Mediated Response Against Daptomycin in Multidrug-Resistant Enterococcus faecalis (Efs)*	2
SANDRA	Rincón	Universidad El Bosque	*Genomic Characterization and Epidemiology of the Cefazolin Inoculum Effect in Methicillin-Susceptible S. aureus from Severe Infections in Patients in Hospitals in Colombia*	2
Jung	Seo	BCM	*Candida Sternal Wound Infections After Cardiac Operations: Uncommon but Deadly*	2
Garima	Singh	UH	*Trick and Treat: Intermittent Antibiotic Dosing to Eradicate Persister Bacteria*	2
Austen	Terwilliger	BCM	*The TAILΦR Initiative at Year One: Personalized Medicine for Dynamic Infections*	2

Poster Presentation Talks Day 2

Luis	Vega	UTH	*The Integrative Conjugative Element ICESpyM92 Contributes to Pathogenicity of Emergent Antimicrobial-Resistant emm92 Group A Streptococcus*	2
Jacob	Zulk	BCM	*Phage Resistance Accompanies Reduced Fitness of Uropathogenic E. Coli in the Urinary Environment*	2

Implementation Outcomes in an Antibiotic Stewardship Program (Kicking CAUTI) in Four Veterans Hospitals Correlated with Clinical Outcomes

Amenta E[1,4], Grigoryan L[1,2] Ramsey D[1,3], Kramer JR[1,3], Walder A,[1,3] Chou A[1,4], Van JN[1], Sales AE[5,6], Naik AD[1,3,7], Trautner BW[1,3]

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Background: One of the major barriers to scale up of antibiotic stewardship interventions is the difficulty of engaging already overtaxed personnel. How to measure implementation of antibiotic stewardship interventions is not well-defined. **Hypothesis/goals**: As part of a multisite antibiotic stewardship dissemination project to decrease overtreatment of asymptomatic bacteriuria (the Kicking CAUTI Campaign), we explored how to measure local implementation efforts, and what dose of the intervention was necessary to achieve a significant improvement in clinical outcomes.

Methods: The intervention was implemented in a staggered manner in 4 different sites from February 2019 through May 2020. We used the Proctor model to choose 3 measures of implementation: the number of intervention delivery sessions (adoption), total number of health care professionals reached (penetration), and minutes spent in delivery of the intervention (adoption). Local site champions kept logs of these activities. The correlation between these implementation measures and the clinical outcomes (the number of urine cultures ordered, days of antibiotic treatment (DOT), and length of antibiotic treatment (LOT)) was calculated using the mixed linear models method.

Results: Site A delivered a total of 156 sessions over 16 months (2/4/2019-5/30/2020) to 643 healthcare professionals for a total time of 2567 minutes in content delivery, averaging 160 minutes/month (**Figure 1**). Site B delivered a total of 240 sessions over 15 months (2/25/2019-5/30/2020) to 798 healthcare professionals for a total of 1465 minutes in content delivery, averaging 97 minutes/month. Site C delivered 49 sessions over 12 months (5/10/2019-5/30/2020) to 542 healthcare professionals for a total of 1317 minutes in content delivery, averaging 109 minutes/month. Site D delivered 45 session over 11 months (6/20/2019-5/30/2020) to 433 healthcare professionals for a total time spent in delivery of 679 minutes in content delivery, averaging 61 minutes/month.

Overall, the minutes spent in delivery ranged from 2567 minutes at the least engaged site to 679 in the most engaged site (**Figure 1**). Minutes spent in delivery was inversely correlated with two of our three clinical metrics, DOT (R -.03, P=0.04) and LOT (R -0.3, P=0.02); significance was not quite met for the correlation of minutes spent and urine cultures (**Table 1**). The number of healthcare professionals reached ranged from 798 to 433, and the number of sessions delivered ranged from 240 to 45. These other implementation metrics did not have a significant relationship with the clinical outcomes.

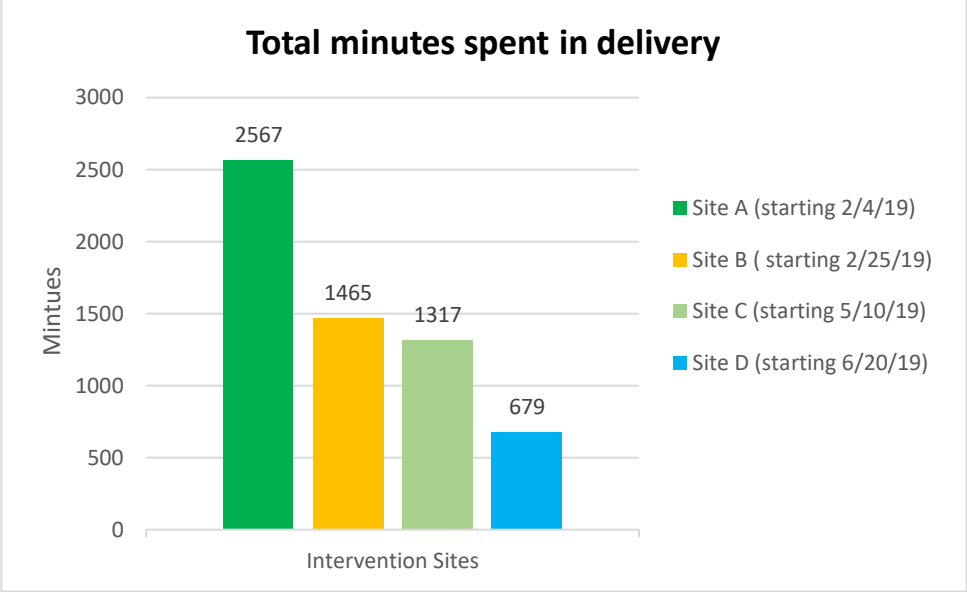
Conclusion: We found a significant negative correlation between the number of minutes a local site champion spent delivering the antibiotic stewardship intervention and antibiotic use, both DOT and LOT. Our implementation metric (adoption) is scalable and readily adaptable to large antibiotic stewardship dissemination projects.

Acknowledgments: VA HSR&D I01HX002171-03 and VA CIN 13-413.

Table 1. Correlation Coefficients (with p-values) Comparing Implementation Outcomes with Clinical Outcomes (**bolded** results are statistically significant)

	Total number of health care professionals reached	Number of intervention delivery sessions	Minutes spent in delivery of the intervention
Urine Cultures	-0.00 (p = 0.99)	- 0.08 (p = 0.61)	- 0.24 (p = 0.10)
Days of therapy (DOT)	- 0.00 (p = 0.98)	- 0.06 (p = 0.69)	**- 0.30 (p = 0.04)**
Length of therapy (LOT)	+ 0.07 (p = 0.62)	- 0.10 (p = 0.49)	**- 0.34 (p = 0.02)**

Figure 1. Total Minutes Spent in Delivery of the Intervention Across Four Intervention Sites



Implementation Outcomes in an Antibiotic Stewardship Program (Kicking CAUTI) in Four Veterans Hospitals Correlated with Clinical Outcomes

Eva Amenta[1,4], Larissa Grigoryan[1,2] David Ramsey[1,3], Jennifer R. Kramer[1,3], Annette Walder,[1,3] Andrew Chou[1,4], John N. Van[1], Anne E. Sales[5,6], Aanand D. Naik[1,3,7], Barbara W. Trautner[1,3]

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Study Design

4 hospitals implemented Antibiotic Stewardship Program

Measured & Performed Mixed Linear Model Methods to assess for correlation

Implementation metrics

- # Providers reached
- # sessions completed
- Total time spent in delivery

Clinical outcomes

- Length of Antibiotics (LOT)
- Days of Antibiotics (DOT)
- Urine Cultures

Results

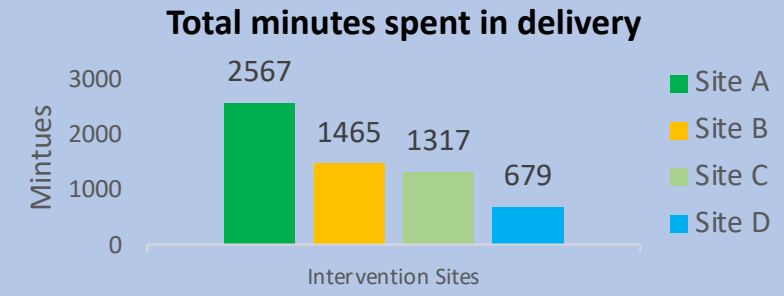

Figure 1. Total Minutes Spent in Delivery of the Intervention Across Four Intervention Sites

Results

Total time spent in delivery

Time spent in delivery was inversely correlated with DOT and LOT

-0.30
p = 0.04
Days of Antibiotics (DOT)

-0.34
p = 0.02
Length of Antibiotics (LOT)

Conclusions

- We found that more implementation of an antibiotic stewardship intervention was associated with decreased use of antibiotics
- We measured implementation by time spent by the local team in delivery of the intervention
- Time spent in delivery of the intervention was inversely correlated with antibiotic use (DOT and LOT)
- Our implementation metric is scalable and readily adaptable to large antibiotic stewardship dissemination projects

Acknowledgements: VA HSR&D I01HX002171-03 and VA CIN 13-413.

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Abstract PDF

SeqScreen: Accurate and Sensitive Functional Screening of Pathogenic Sequences via Ensemble Learning

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Background Modern benchtop DNA synthesis techniques and increased concern of emerging pathogens have elevated the importance of screening oligonucleotides for pathogens of concern. However, accurate and sensitive characterization of oligonucleotides is an open challenge for many of the current techniques and ontology-based tools. This is especially true in case of near-neighbor pathogens (>90% ANI) which are commonly misclassified by k-mer or DNA alignmment based tools associated with taxonomic labels.

Hypothesis/Goals We reasoned that using functional labels to detect specific pathogenic markers was a more reliable marker of pathogenic potential of a sequence. Genomes are dynamic and over time may lose or acquire certain genes associated with pathogenicity which might not be reflected in the reference databases and hence relying on taxonomic label might not be optimal. From an emerging pathogen perspective, we know that amino acids are more conserved than DNA sequences and hence by using a protein database we might be able to detect more distant pathogenic proteins that might be missed at the DNA level. Finally, we hypothesized that training on proteins using Gene Ontology terms, keywords as features would enable us to create robust predictive models that could help automate capture of a diverse variety of pathogenic markers. In order to classify the markers into distinct groups based on the mechanism of pathogenicity, our biocurators introduced 32 distinct Functions of Sequences of Concern (FunSoCs) including antimicrobial resistance.

Methods SeqScreen pipeline consists of two modes default and sensitive. The modes mainly differ in the tool use to align sequences to the protein database. The default mode uses DIAMOND whereas the sensitive mode uses BLASTX which is slower but more sensitive. To improve the efficiency of assigning FunSoC labels to sequences, we include a FunSoC database with SeqScreen that contains pre-predicted labels for all the proteins in our database. The predictions were carried out by an ensemble classifier comprising of three top performing machine learning models that comprise of feature selection methods as well as multi-task models. We measure the performance of various models across eight different metrics to identify these top models.

Results To address this gap, we have developed a novel software tool, SeqScreen, that can accurately and sensitively characterize short DNA sequences using a set of curated Functions of Sequences of Concern (FunSoCs), novel functional labels specific to microbial pathogenesis which describe the pathogenic potential of individual proteins. We show that our ensemble machine learning model after training on these curations can label sequences with FunSoCs via an imbalanced multi-class and multi-label classification

task with high accuracy. Further, we show that SeqScreen can detect the presence of emerging pathogens from metatranscriptomes.

Conclusion In summary, SeqScreen represents a first step towards a novel paradigm of functionally informed pathogen characterization from genomic and metagenomic datasets. SeqScreen is open-source and freely available for download at: www.gitlab.com/treangenlab/seqscreen

Acknowledgements All of the co-authors were either fully or partially supported by the Fun GCAT program from the Office of the Director of National Intelligence (ODNI), Intelligence Advanced Research Projects Activity (IARPA), via the Army Research Office (ARO) under Federal Award No. W911NF-17-2-0089. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the ODNI, IARPA, ARO, or the US Government. L.E. was partially supported by a training fellowship from the Gulf Coast Consortia, on the NLM Training Program in Biomedical Informatics & Data Science (T15LM007093).

SeqScreen: Accurate and Sensitive Functional Screening of Pathogenic Sequences via Ensemble Learning

Advait Balaji[1], Bryce Kille[1], Anthony D. Kappell[3], Gene D. Godbold[3], Madeline Diep[4], R. A. Leo Elworth[1], Zhiqin Qian[1], Dreycey Albin[1], Daniel J. Nasko[5], Nidhi Shah[5], Mihai Pop[5], Santiago Segarra[2], Krista L. Ternus[3], Todd J. Treangen[1]

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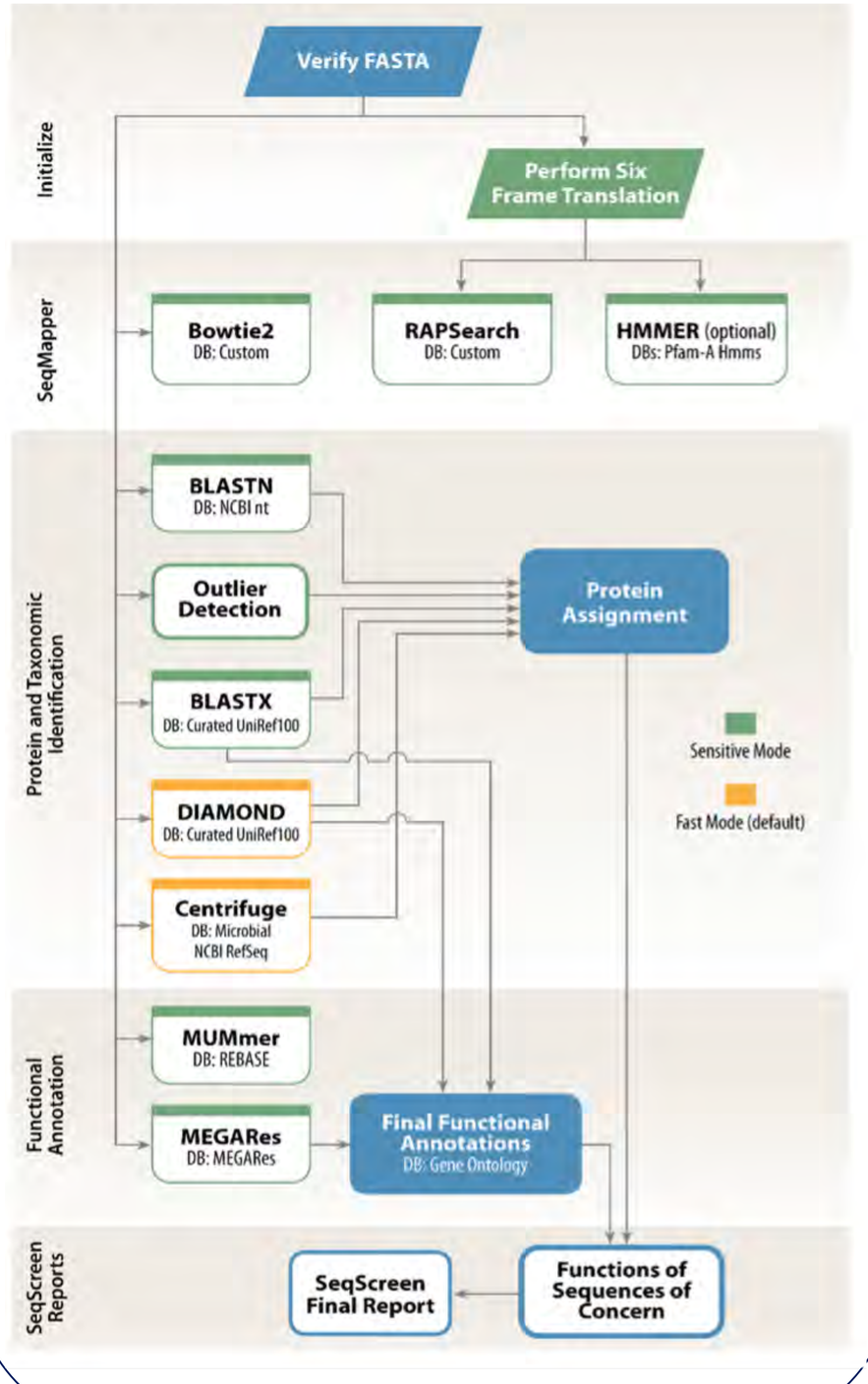
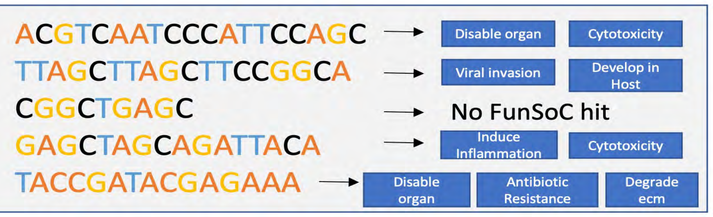
PIPELINE

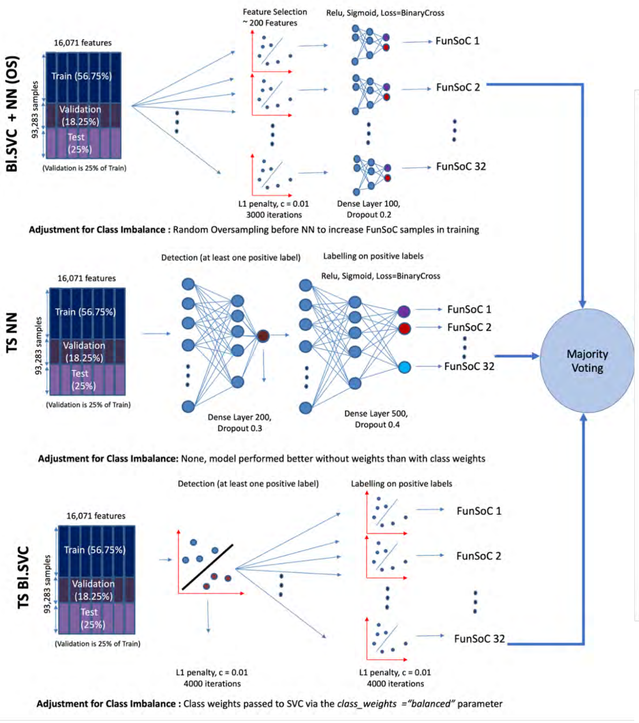


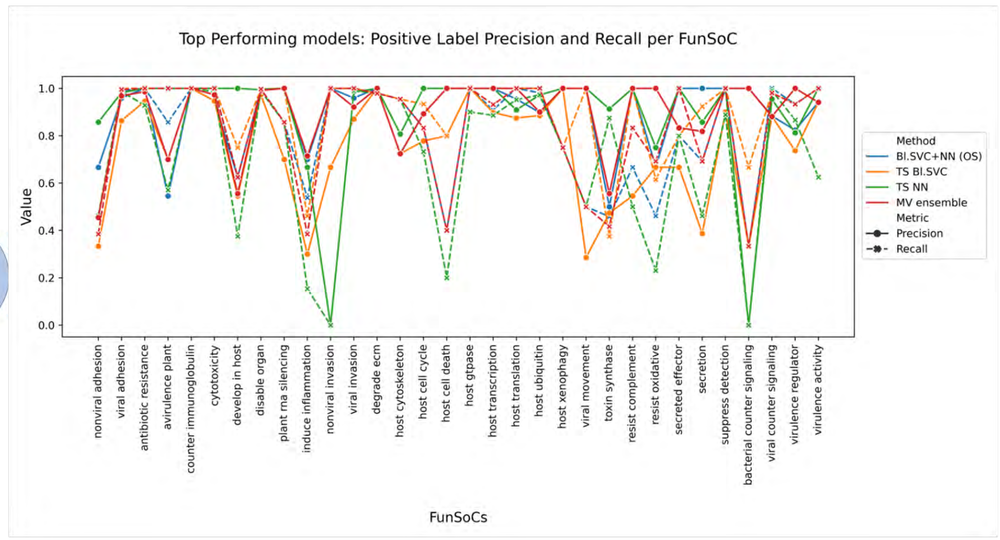
TASK

Assign short DNA sequences with pathogenic labels
FunSoCs (Functions of sequences of concern)



FunSoC ML Pipeline





DISTINGUISHING NEAR-NEIGHBOR PATHOGENS

FunSoCs

Bacteria: Isolate genome (SRA accession)	disable organ	cytotoxicity	degrade ecm	induce inflammation	secreted effector	secretion	antibiotic resistance	bacterial counter signaling	counter immunoglobulin	virulence regulator
(a) *E.coli K12 MG1655* (DRR198806)		*hlyE*			*tir*	17 genes identified*	35 genes identified*			
(b) *E.coli O157:H7* (DRR198804)		*stxB, hlyE*			*espF(U), tir*	15 genes identified*	34 genes identified*			*espF(U),nleF*
(c) *C.sporogenes* (SRR8758382)						*T3SA*	*bla, uppP, pbpA*	*moaC*		
(d) *C.botulinum* (SRR8981313)	*botA*	*orf-X2*	*botA*				*bla, uppP, pbpA*	*moaC*		*botA*
(e) *S.dysgalactiae* (SRR12825903)		*SLO*				*comYC*	*uppP, pilA*		*spg*	
(f) *S.pyogenes* (ERR1735064)		*SLO*		*speB, speH*		*comYC*	*uppP*			
(g) *S.salivarius* (SRR11910125)						*cglC, comGC*	*mprF, uppP, pbpA*			
(h) *L.gasseri* (SRR11910134)							*mprF, aac(3)*			

The Role and Dynamics Of Ethanolamine-Utilizing Bacterial Microcompartments

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Background: Bacterial pathogens face many challenges within the host environment including nutrient starvation. Therefore, finding alternative nutrient and fuel sources is of key importance. Ethanolamine (EA) is a potential source of carbon, nitrogen and/or energy in host environments for many bacteria including species of *Enterococcus*, *Escherichia*, *Clostridium*, *Listeria*, *Klebsiella*, and *Salmonella*. In common, these bacteria have a *eut* (ethanolamine utilization) locus that consists of the genes required for EA metabolism.

Hypothesis: Catabolism of EA takes place inside special microcompartments known as bacterial microcompartments (BMC), which are viral capsid like proteinaceous icosahedral structures. In this study using *Listeria monocytogenes* as a model organism, the importance and dynamics of BMC formation was examined with the hypothesis that it is a nutrient source that contributes to intracellular replication.

Methods: A nitrogen free minimal media (ACMM) was optimised for this study. Bacterial growth was monitored in ACMM in the presence and absence of EA until the cells reached stationary phase. qRT PCR was done to investigate the expression of the *eut* genes in presence and absence of EA. A recombinant *L. monocytogenes* was generated that expressed the BMC structural gene *eutK* as a mNeongreen (mNG) fusion protein. The strain was used to visualize the formation of BMC using confocal imaging. Mutants lacking the response regulator EutV, necessary for activating *eut* gene expression, and ammonia lyase EutB, necessary for first step in the catabolism of EA, were also generated and compared to wild type. Bone marrow derived macrophages (BMDM) were infected with the wild type and mutant strains, to identify any growth defects pertaining to BMC formation and EA metabolism.

Results: *L. monocytogenes* was able to grow on a nitrogen free minimal media (ACMM) only when EA or glutamine was added as a nitrogen source. On the other hand, *eutV* and *eutB* knockout strains were not able to utilize EA as a nitrogen source and did not grow. Addition of glutamine as a nitrogen source rescued the growth defect in the knockout strains. qRT PCR analysis showed that the *eut* genes were upregulated upon addition of EA to the ACMM media. Confocal imaging of the recombinant bacteria expressing eutK-mNG showed the formation of BMC like structures in presence of EA, which was greatly reduced in the *eutV* knockout strain. The eutV and eutB knockout strains were defective of intracellular replication in BMDM.

Conclusions: The study confirms the hypothesis that in the model organism *L. monocytogenes*, EA can be used as a nutrient source, specifically of nitrogen, and that this metabolism contributes to optimal intracellular replication. Additionally, it provides preliminary evidence that the Eut BMCs of *L. monocytogenes* can be fluorescently tagged, providing a tool for future studies in host cells.

Acknowledgement: This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number R21AI167124 to DAG.

The Role And Dynamics Of Ethanolamine-Utilizing Bacterial Microcompartments

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INTRODUCTION/ABSTRACT

Bacterial pathogens face many challenges within the host environment including nutrient starvation. Therefore, finding alternative nutrient and fuel sources is of key importance. Ethanolamine (EA) is a potential source of carbon, nitrogen and/or energy in host environments for many bacteria including species of *Enterococcus*, *Escherichia*, *Clostridium*, *Listeria*, *Klebsiella*, and *Salmonella*. In common, these bacteria have a *eut* (ethanolamine utilization) locus that consists of the genes required for EA metabolism.

Catabolism of EA takes place inside special microcompartments known as bacterial microcompartments (BMC), which are viral capsid like proteinaceous icosahedral structures. In this study using *Listeria monocytogenes* as a model organism, the importance and dynamics of BMC formation was examined with the hypothesis that it is a nutrient source that contributes to intracellular replication.

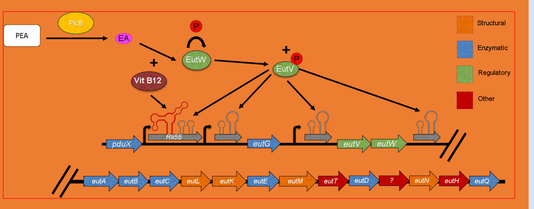

Figure 1. *eut* locus in *Listeria monocytogenes*

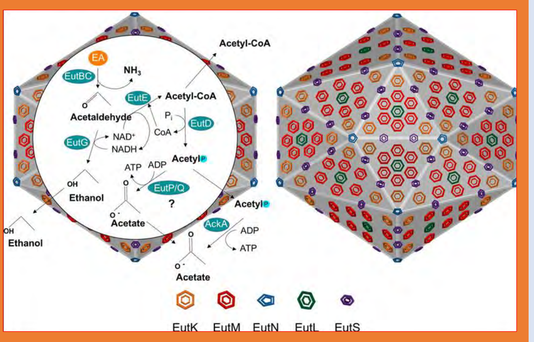

Figure 2. Graphical representation of BMC structure and function.
Karan Gautam Kaval, Danielle A. Garsin

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MATERIALS AND METHODS

A nitrogen free minimal media (ACMM) was optimised for this study. Bacterial growth was monitored in ACMM in the presence and absence of EA until the cells reached stationary phase. qRT PCR was done to investigate the expression of the *eut* genes in presence and absence of EA. A recombinant *L. monocytogenes* was generated that expressed the BMC structural gene *eutK* as a mNeongreen (mNG) fusion protein. The strain was used to visualize the formation of BMC using confocal imaging. Mutants lacking the response regulator EutV, necessary for activating *eut* gene expression, and ammonia lyase EutB, necessary for first step in the catabolism of EA, were also generated and compared to wild type. Bone marrow derived macrophages (BMDM) were infected with the wild type and mutant strains, to identify any growth defects pertaining to BMC formation and EA metabolism.

RESULTS

L. monocytogenes was able to grow on a nitrogen free minimal media (ACMM) only when ethanolamine (EA) or glutamine (Gln) was added as a nitrogen source. On the other hand, *eutV* and *eutB* knockout strains were not able to utilize EA as a nitrogen source and did not grow. qRT PCR analysis showed that the *eut* genes were upregulated upon addition of EA to the ACMM media. Confocal imaging of the recombinant bacteria expressing eutK-mNG showed the formation of BMC like structures only in presence of EA and not Gln. The *eutV* and *eutB* knockout strains were defective of intracellular replication in BMDM. Confocal imaging of J774A.1 cell line infected with recombinant *L. monocytogenes* expressing *eutK-mNG* also showed the formation of BMC like structures *Ex vivo.*

Components	Molar
Na2HPO4.7H2O	11.55 mM
KH2PO4	4.82 mM
MgSO4.7H2O	1.7 mM
Glucose	55 mM
Methionine	0.67 mM
Cysteine	0.82 mM
Riboflavin	1.33 uM
Thiamine	2.96 uM
Biotin	2.05 uM
Lipoic acid	24 pM
MOPS pH 7.4	100 mM
Leucine	0.76 mM
Isoleucine	0.76 mM
Valine	0.85 mM
Methionine	0.67 mM
Arginine	0.57 mM
Histidine	0.64 mM
Cysteine	0.82 mM
Glutamine	4.1mM

Table 1. Composition of the minimal media (ACMM)

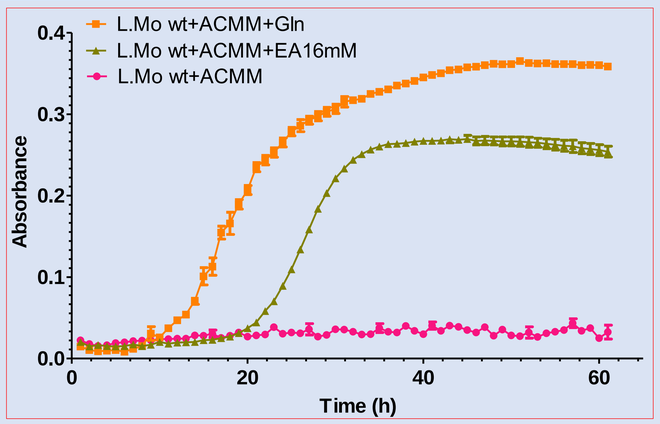

Figure 3. Growth of *L. monocytogenes* in ACMM containing Glutamine (Gln) or Ethanolamine (EA) as nitrogen source.

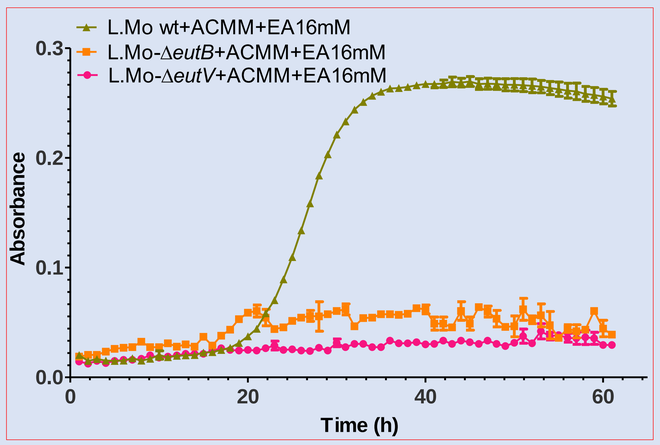

Figure 4. Growth of *L. monocytogenes* wild type, *ΔeutB* and *ΔeutV* in ACMM and Ethanolamine (EA) as nitrogen source.

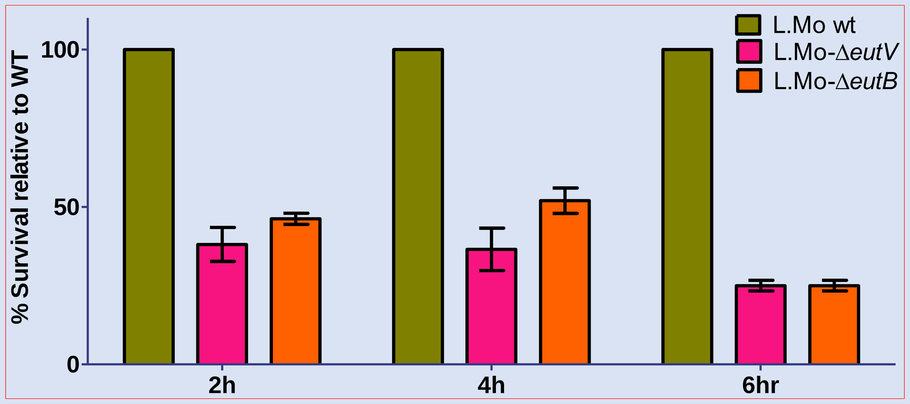

Figure 5. Survival of *L. monocytogenes wild type, ΔeutB and ΔeutV* in BMDM. MOI 5, 30 min infection.

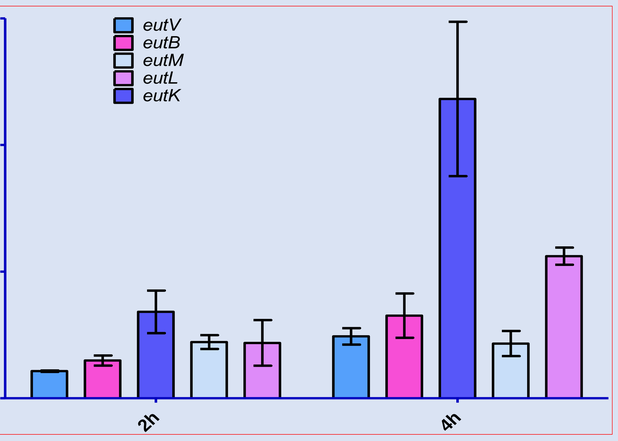

Figure 6. Relative expression of various genes at 2h and 4h when cells were grown in ACMM containing ethanolamine (EA) as nitrogen source with respect to cells grown in ACMM containing glutamine (Gln) as a nitrogen source.

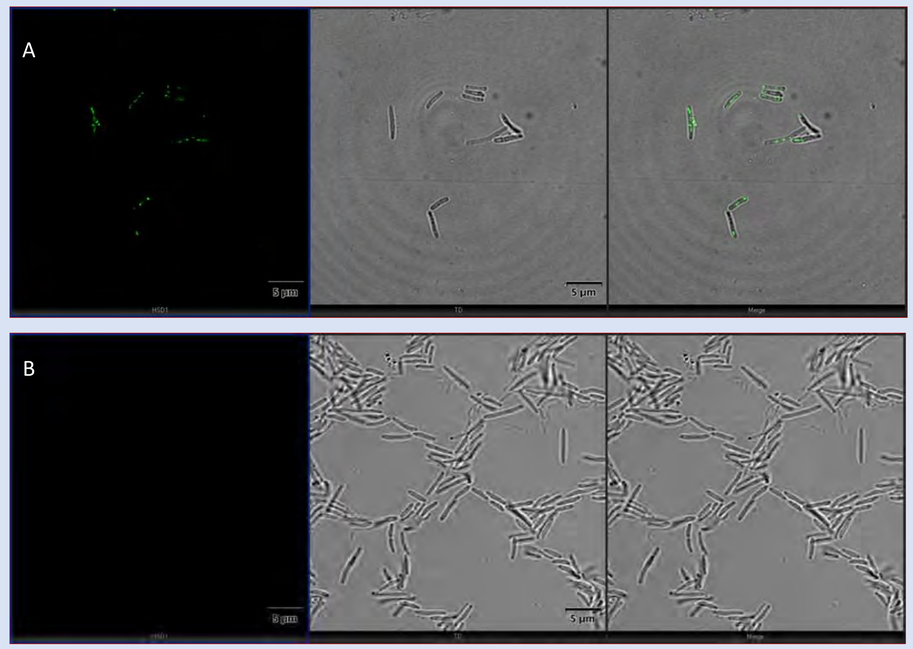

Figure 7. (A) Confocal imaging of *L. monocytogenes* showing expression of structural gene *eutK* tagged with mNeonGreen when grown in ACMM containing ethanolamine, and (B) when the cells were grown in ACMM containing glutamine as a nitrogen source.

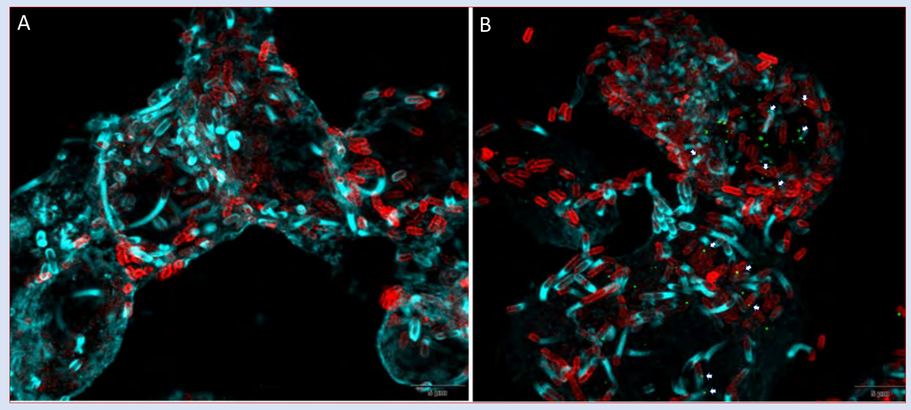

Figure 8. Confocal imaging showing *L. monocytogenes* inside J774A.1 cell line. (A) *L. monocytogenes* wild type control. (B) *L. monocytogenes* expressing structural protein *eutK* tagged with mNeonGreen under the control of *eutV* promoter. Blue = actin staining with phalloidin, Red = anti-listeria antibody and Green = *eutK*-mNeonGreen.

CONCLUSIONS

The study confirms the hypothesis that in the model organism *L. monocytogenes*, ethanolamine can be used as a nutrient source, specifically of nitrogen, and that this metabolism contributes to optimal intracellular replication. Additionally, it provides preliminary evidence that the Eut BMCs of *L. monocytogenes* can be fluorescently tagged, providing a tool for future studies in host cells.

ACKNOWLEDGEMENT

This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number R21AI167124 to DAG.

Increased Virulence Potential Among Antimicrobial Resistant Coagulase-Positive Staphylococci Associated with Animal Pyoderma

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* Contributed equally to this work

Background: Coagulase-positive staphylococci (CoPS) account for most bacteria-related pyoderma in pets. Emergence of methicillin-resistant *S. pseudintermedius* (MRSP), methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug resistant (MDR) strains in pets is a growing public health concern.

Goals: The study aimed to (i) characterize pyoderma-related CoPS regarding biofilm production relating this trait with circulating clonal lineages and antibiotic resistance; and to (ii) evaluate their virulence potential in a *Galleria mellonella* infection model.

Materials: The study comprised 155 *S. pseudintermedius* and 55 *S. aureus* collected in Lisbon, Portugal from pets (dogs, cats, rabbits) previously characterized regarding resistance patterns and clonal lineages. Biofilm production was evaluated by the crystal violet adhesion method. The virulence potential of CoPS strains of interest (predominant clonal lineages, biofilm^{+}, MRSP/MRSA) was evaluated in a *G. mellonella* infection model and compared to strains *S. aureus* RN4220 and *S. pseudintermedius* DSM21284^{T}.

Results: Biofilm production was a common trait of *S. pseudintermedius* (33.6%, 52/155) and *S. aureus* (71%, 39/55) strains, highly associated with the predominant circulating clonal lineages, namely MRSP-MDR-ST71-*agrIII*, MRSA-MDR-ST22-*agrI*, MRSA/MSSA-ST5-agrII and MSSA-ST398-*agrI*. Kaplan-Meier survival analysis of *G. mellonella* revealed the virulence potential of pyoderma-related MRSP-MDR strains belonging to ST71-*agr*III or ST118-*agr*II and, notably of MRSA-MDR-ST22-*agr*I strains.

Conclusions: This study highlights a high frequency of biofilm production by prevalent antimicrobial-resistant clonal lineages of *S. pseudintermedius* and *S. aureus* associated with animal pyoderma, potentially related with a higher virulence potential. These findings strengthen the need for a One Health approach in the evaluation of the health hazard potential of antimicrobial resistant CoPS causing infections in pets.

Ackowledgements: Project BIOSAFE funded by FEDER (Grant LISBOA-01-0145-FEDER-030713) and FCT (Grant PTDC/CAL-EST/30713/2017). Further support by FCT to GHTM (UID/04413/2020); CIISA-UID/CVT/00276/2020 and UI/BD/151061/2021 (CM).

Increased virulence potential of biofilm-producing coagulase-positive staphylococci associated with animal pyoderma

Mariana Andrade[1,*], Ketlyn Oliveira[1,*], Catarina Morais[1], Patrícia Abrantes[1], Constança Pomba[2,3], Adriana E. Rosato[4,5], Isabel Couto[1], and Sofia Santos Costa[1]

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* Contributed equally to this work

5th Annual Texas Medical Center Antimicrobial Resistance & Stewardship Conference

January 19-21, 2022

INTRODUCTION

- ***Staphylococcus pseudintermedius*** **is the main bacterial agent of pyoderma in pets, particularly dogs[1], while *S. aureus* is a less frequent yet relevant agent of pyoderma in cats and dogs[2].**
- **The increasing frequency of methicillin-resistant *S. pseudintermedius* (MRSP) and methicillin-resistant *S. aureus* (MRSA), frequently associated with multidrug resistance (MDR), is a public health concern[3].**

GOALS

- **Characterize biofilm production in coagulase-positive staphylococci (CoPS) causing pyoderma, relating this trait with clonal lineages and antibiotic resistance;**
- **Evaluate their virulence potential in a *Galleria mellonella* infection model.**

METHODOLOGY

155 *S. pseudintermedius* (SP)*

Lisbon, 2014 - 2018

141 🐕 3 🐈 1 🐇

55 *S. aureus* (SA) *

Lisbon, 2001-2018

27 🐕 19 🐈 5 🐇 1 🐎 1 unknown

Reference strains
S. pseudintermedius DSM21284^T
S. aureus RN4220

* Previously characterized for antibiotic resistance and clonal lineages

Evaluation of biofilm production [4,5]

Crystal violet adhesion assay

- cell growth in TSB + 1% glucose + / - NaCl
- measurement of total biomass at OD_{570} in a Synergy HT apparatus (Biotek)
- Biofilm phenotypes: categorized according to modified Stepanović criteria

$OD_{strain} \leq 2xODc$ → **Non-producer**
$OD_{strain} > 2xODc$ → **Producer**


G. mellonella infection model

Evaluation of virulence potential of 3 biofilm-positive representative strains

- **BIOS-V64**: MRSP-MDR-ST71-*agrIII*
- **BIOS-V262**: MRSP-MDR-ST118-*agrII*
- **BIOS-V204**: MRSA-MDR-ST22-*agrI*

- two reference strains; controls (PBS, no manipulation)
- inoculation w/ 10^5 and 10^7 CFU/larva
- 10 larvae/group, 37 °C, survival verified each 24h post-infection for 7 days

RESULTS

Biofilm production and its relation with antibiotic resistance and clonal lineages

- **High frequency of biofilm production among animal-associated CoPS, associated with the main circulating clonal lineages**

S. pseudintermedius
Overall: 33.6 % (52/155)
MRSP-MDR-ST71-*agrIII:* 54% (13/24)

S. aureus
Overall: 70.9 % (39/55)
MRSA-MDR-ST22-*agrI:* 60% (15/25)
MRSA/MSSA-ST5-*agrII:* 86% (6/7)
MSSA-ST398-*agrI:* 80% (4/5)

- **NaCl can act as an inducer or inhibitor of biofilm production in both species (Fig 1).**

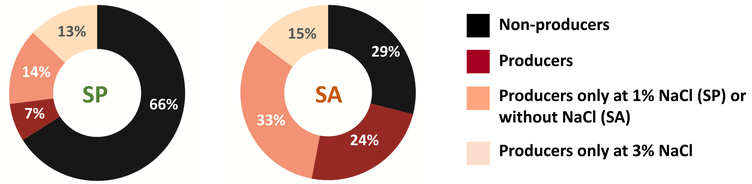
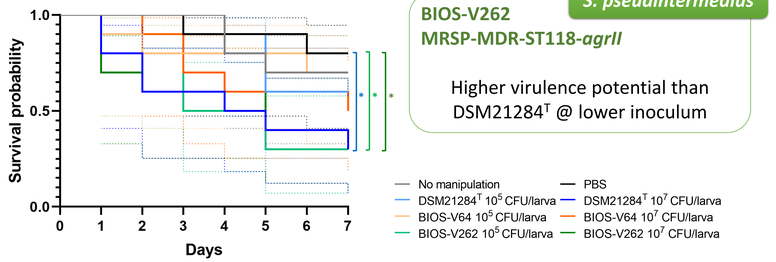


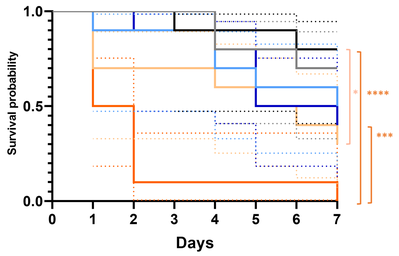
Fig 1 . Biofilm production by *S. pseudintermedius* (n = 155) and *S. aureus* (n = 55) strains.

Assessment of virulence potential of biofilm-producing CoPS



S. pseudintermedius
BIOS-V262
MRSP-MDR-ST118-*agrII*

Higher virulence potential than DSM21284^T @ lower inoculum



S. aureus
BIOS-V204
MRSA-MDR-ST22-*agrI*

Higher virulence potential than RN4220 and *S. pseudintermedius* strains @ higher inoculum

Fig 2 . Kaplan-Meier survival analysis of *G. mellonella* infected with 3 biofilm producing CoPS representative of the main clonal lineages causing animal pyoderma. The colored dotted lines indicates the 95% confidence interval for the corresponding survival curve. Statistical diferences are highlighted as follows: * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$. Analysis performed in GraphPad prism v8.0.1.

CONCLUSIONS

- **Biofilm production is a frequent trait of antimicrobial resistant CoPS causing pyoderma in pets, particularly *S. pseudintermedius* ST71-*agrIII* and *S. aureus* ST22-*agrI*, clonal lineages prevalent in Portugal and other European countries.**
- **Increased virulence of *S. aureus* ST22-*agrI* and, in lesser extent, *S. pseudintermedius* ST118-*agrII* (SLV of ST258, a clone replacing ST71 in several European countries).**

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ACKNOWLEDGEMENTS

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An Epidemiologic Exploration of Vancomycin Resistance in Clostridioides difficile

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Background
Although *Clostridioides difficile* infection (CDI) is the most common healthcare-associated infection in the United States, vancomycin is one of only three antibiotics available to treat CDI. However, vancomycin clinical cure rates have decreased since the early 2000's to 70-80% in recent randomized controlled trials. Additionally, vancomycin use has increased by 54% following recent treatment guideline updates, applying significant antibiotic resistance selection pressure. As susceptibility testing is not routinely performed in *C. difficile*, the clinical significance of vancomycin resistance is not well understood. The aim of this exploratory study was to investigate the prevalence, risk factors, and outcomes associated with vancomycin resistance in clinical samples from the Texas Medical Center.

Methods
This multicenter cohort study included adult hospitalized patients with CDI between 2017-2020. Samples were transported to our centralized lab, plated onto selective cefoxitin-cycloserine-fructose agar (CCFA) plates, and anaerobically incubated for 48–72 hours for culture. Fluorescent PCR ribotyping was performed and vancomycin minimum inhibitory concentration (MIC) testing was conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI) standards. Vancomycin resistance was defined as MIC > 2 mg/L as per CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidance. An exploratory analysis of patients receiving vancomycin treatment was conducted in which patients infected with vancomycin-resistant strains were compared to those infected with wild-type strains (MIC ≤ 2 mg/L). Descriptive statistics were assessed using SPSS (version 27.0.0.0).

Results
Of 179 isolates analyzed, 9 (5%) were resistant to vancomycin (> 2 mg/L). Vancomycin MICs ranged from 0.5 to >16 mg/L, with an MIC50 of 1 mg/L and an MIC90 of 2 mg/L. Ribotypes displaying resistance included F014-020, F027, F106, F116, and FP318; no single ribotype was correlated with increased resistance. Two of six patients with a resistant isolate and initially treated with vancomycin exhibited treatment failure at day 6.

Conclusion
Around 5% of *C. difficile* in the Texas Medical Center demonstrated vancomycin resistance. Future research directions include an expansion of the cohort and further correlation of this resistant phenotype with clinical outcomes.

Funding
This project was funded by the National Institute of Allergy and Infectious Diseases (NIAID), T32 AI141349.

An Epidemiologic Exploration of Vancomycin Resistance in *Clostridioides difficile*

Eubank TA[1], Gonzales-Luna AJ[1], Costa G[1], Loveall C[1], Lancaster C[1], Hurdle JG[2], Garey KW[1]

[1]Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy; [2]Center for Infectious and Inflammatory Diseases, Texas A&M Health Science Center

BACKGROUND

- Although *Clostridioides difficile* infection (CDI) is the most common healthcare-associated infection in the United States, vancomycin is one of only three antibiotics used to treat CDI[1]
- Clinical cure rates with vancomycin have decreased since the early 2000's to ~80% in recent randomized controlled trials[2,3]
- Vancomycin use has increased by 54% following 2018 IDSA/SHEA treatment guideline updates, applying significant antibiotic resistance selection pressure[4]
- As susceptibility testing is not routinely performed in *C. difficile*, the clinical significance of vancomycin resistance is not well understood

OBJECTIVES

- To investigate the prevalence of vancomycin resistance in clinical samples from the Texas Medical Center

METHODS

Study design / Inclusion

- Multicenter cohort study
- Adult hospitalized patients with CDI between 2017 – 2020

Statistical analysis

- Descriptive statistics were assessed using SPSS (version 27.0.0.0)

Sample processing / Microbiology:

- Discard stool samples transported from the Texas Medical Center to our centralized lab at the University of Houston College of Pharmacy
- Stool plated onto selective cefoxitin-cycloserine-fructose agar (CCFA) plates and anaerobically incubated for 48 – 72 hours for culture
- Fluorescent PCR ribotyping conducted as previously described
- Vancomycin MIC testing conducted via agar dilution in accordance with CLSI standards

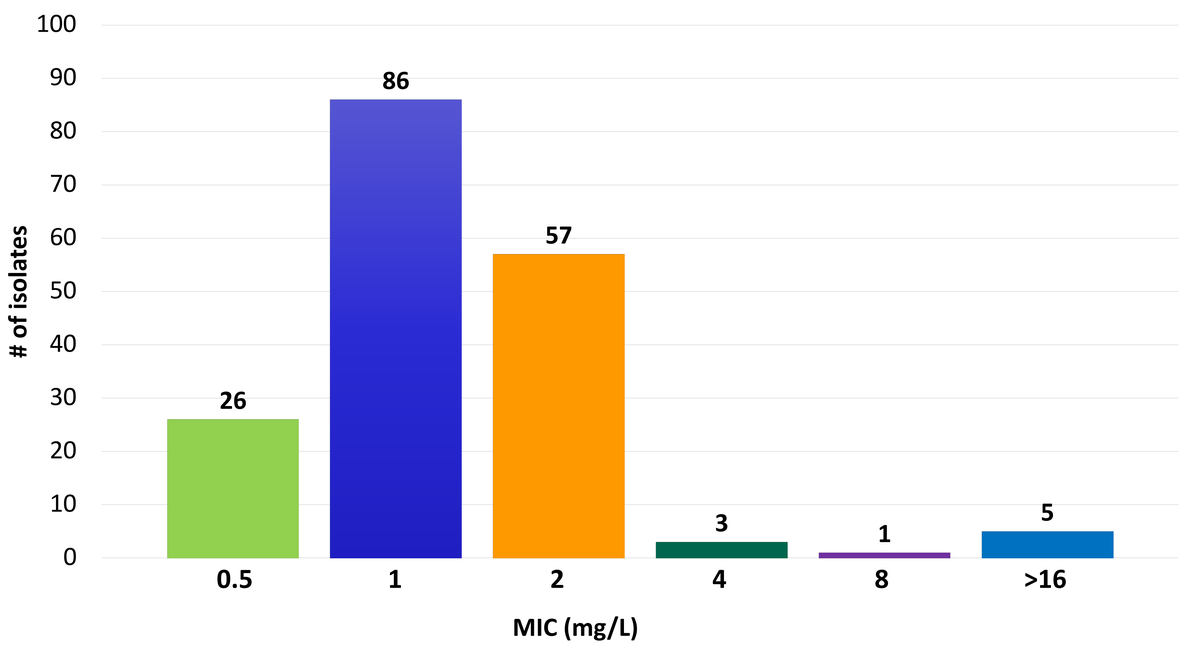
RESULTS

- Total sample included 178 clinical isolates
  - 9 isolates (5%) demonstrated vancomycin resistance
  - Vancomycin MICs ranged from 0.5 to >16 mg/L, with MIC_{50} = 1 mg/L and MIC_{90} = 2 mg/L
- F014-020 was the most common ribotype overall and in those with vancomycin resistance (Table 2)

Table 1. Vancomycin susceptibility by ribotype (n = 178)

Ribotype	# isolates	MIC_{50}	MIC_{90}	MIC range	% resistant
All	178	1 mg/L	2 mg/L	0.5 - >16 mg/L	5.06%
F014-020	73	1 mg/L	2 mg/L	0.5 - >16 mg/L	4.1%
F027	40	2 mg/L	2 mg/L	0.5 - 4 mg/L	5.0%
F106	26	1 mg/L	2 mg/L	0.5 - >16 mg/L	7.7%
F002	9	1 mg/L	2 mg/L	1 - 2 mg/L	0%
F103	3	1 mg/L	1 mg/L	1 mg/L	0%
Other	27	1 mg/L	2 mg/L	0.5 - >16 mg/L	7.4%

Figure 1. Vancomycin MIC distribution (n = 178)



DISCUSSION

- Rates of vancomycin resistance in the Texas Medical Center are similar to those reported in surveillance studies[5]
- F014-020 was the most prevalent ribotype demonstrating vancomycin resistance, in contrast with reports that F027 has more vancomycin resistance[6]
- Several genetic mechanisms of vancomycin resistance have been previously described including cell wall alterations, efflux pumps, and biofilm production
- Future genetic analysis of these resistant isolates will be pertinent to advances in combatting negative clinical outcomes

CONCLUSION

- Around 6% of *C. difficile* in the Texas Medical Center demonstrated vancomycin resistance, which did not correlate with treatment failure in an exploratory analysis.
- Future research directions include an expansion of the cohort and further correlation of this resistant phenotype with clinical outcomes

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Using Whole Genome Sequencing to Genetically Profile and Analyze Escherichia coli Isolates with Varying Resistance to β-lactam/β-lactamase Inhibitor Combinations

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Background: Whole-genome sequencing has garnered a tremendous amount of interest as an avenue for assessing antimicrobial resistance (AMR). However, multiple studies have shown a poor correlation between the β-lactam/β-lactamase inhibitor (BL/BLI) phenotype and β-lactamase gene presence/absence. In a recent publication, we identified that amplification of β-lactamase encoding genes could potentially contribute to BL/BLI resistance. Hence, we sought to determine whether consideration of gene amplification would allow for better BL/BLI genotype/phenotype correlation.

Methods: We selected 109 *E. coli* bacteremia isolates from MD Anderson Cancer Center (MDACC) which had previously been characterized by the clinical microbiology laboratory as being susceptible to ceftriaxone but with varying BL/BLI antimicrobial susceptibility (AST) profiles. ETest (bioMerieux, Inc) was used to confirm susceptibility profiles to the following BL/BLI antibiotics: ampicillin/sulbactam (SAM), amoxicillin/clavulanic acid (AMC), and piperacillin/tazobactam (TZP). CLSI M100 guidelines (2018) were used to stratify isolates into 4 groups: SAM/AMC/TZP susceptible (Group 1), SAM resistant only (Group 2), SAM/AMC resistant (Group 3), and SAM/AMC/TZP resistant (Group 4). Short-read whole genome sequencing (WGS) was performed using Illumina NovaSeq6000 to provide a genomics analysis, in particular, identify AMR genes (ABRicate, T Seemann: GitHub: https://github.com/tseemann/abricate), establish multi-locus sequence type (MLST) (mlst, T Seemann: GitHub: https://github.com/tseemann/mlst), and estimate copy number variants (CNV) (convict, W Shropshire: GitHub: https://github.com/wshropshire/convict). A t-Distributed Stochastic Neighbor Embedding (t-SNE) clustering method was employed with CNV data as an input to determine if any isolates clustered based on their respective AMR gene content and CNV data.

Results: We identified 34 different MLSTs with the three most common sequence types being ST131 (n=41), ST1193 (n=12), and ST648 (n=6). All ST648 (n=6) were resistant to SAM/AMC/TZP (Group 4) while 66.7% of ST1193 bacteremia isolates had SAM resistance only (8/12; 66.7%; Group 2). Group 1 and Group 2 isolates had similar mean copy number estimates of the narrow spectrum β-lactamase $bla_{\text{TEM-1B}}$ (1.8 and 1.5 respectively) while there was a substantial increase for both Group 3 isolates (3.7) and Group 4 (8.2) isolates respectively (Table 1). Additionally, $bla_{\text{OXA-1}}$ was almost uniquely present in Group 4 isolates (17/18 cases) typically with elevated copy numbers (mean 5.2). Our t-SNE analysis showed that fully susceptible isolates (Group 1) and fully resistant isolates (Group 4) cluster independently.

Conclusions: There is a clear delineation between fully susceptible and fully resistant BL/BLI *E. coli* isolates when gene amplification is considered. Furthermore, an increase in copy number of narrow spectrum β-lactamases is observed when looking across each of the four BL/BLI group designations. Narrow spectrum β-lactamase amplifications need to be considered for BL/BLI resistance prediction models.

Table 1: Narrow Spectrum β-lactamase Characteristics Across BL/BLI Group Spectrum

	Group 1 (n=26)	Group 2 (n=28)	Group 3 (n=28)	Group 4 (n=27)
$bla_{\text{TEM-1B}}$ (n; %)	13 (50)	27 (96.4)	26 (92.9)	15 (55.6)
Copy Number (mean; sd)	1.8 (0.7)	1.5 (0.7)	3.7 (4.5)	8.2 (12.1)
$bla_{\text{OXA-1}}$ (n; %)	0 (0)	0 (0)	1 (3.6)	17 (63.0)
Copy Number (mean; sd)	NA	NA	0.8 (NA)	5.2 (4.8)

Using whole genome sequencing to genetically profile and analyze *Escherichia coli* isolates with varying resistance to β-lactam/β-lactamase inhibitor combinations

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Introduction

Beta lactams in conjunction with serine beta-lactamase inhibitors are widely used to combat bacterial infections. Past study on the use of whole genome sequencing (WGS) to predict resistance patterns in gram-negative bacteria has shown that amongst the most commonly used antibiotics, the beta lactam-beta lactamase inhibitor (BL/BLI) combinations have shown considerably less specificity than the study's counterparts, including ceftazidime, cefepime, and meropenem. This study focuses on three major BL/BLI combinations with different susceptibilities to compare the variants and copy numbers associated with antimicrobial resistance genes, to determine whether gene amplification would allow for better genotype/phenotype correlation. Our primary focus is on genes that are involved in the production of enzymes capable of hydrolyzing these extended spectrum beta lactam antibiotics, such as TEM-1, OXA-1, CARB-3, CTX-M, and TEM variants. Furthermore, we aim to use t-distributed stochastic neighbor embedding and principal component analysis to genetically characterize the use of ampicillin/sulbactam, amoxicillin/clavulanic acid, and piperacillin/tazobactam in four unique combination groups of susceptibility and resistance.

Methods

One hundred and nine bacteremia isolates of E. Coli are obtained from blood samples of patients at the MDACC Hospital in Houston, TX. Antimicrobial susceptibility profiles for these isolates are acquired from the clinical microbiology lab via ETest (bioMerieux, Inc). Minimum inhibitory concentration data for these isolates is then used to divide our sample into four unique groups based on their resistance to BL/BLI combinations- ampicillin/sulbactam(SAM), amoxicillin/clavulanic acid(AMC), and piperacillin/tazobactam(TZP). CLSI M100 guidelines (2018) were used to stratify isolates into 4 groups:

- SAM/AMC/TZP susceptible (Group 1)
- SAM resistant only (Group 2)
- SAM/AMC resistant (Group 3)
- SAM/AMC/TZP resistant (Group 4)

Table 1: characterization of bacteremia isolates into four groups

	Ampicillin/ Sulbactam (SAM)	Amoxicillin/ clavulanic acid (AMC)	Piperacillin/ tazobactam (TZP)	Number of Isolates
Group 1	S	S	S	26
Group 2	R	S	S	28
Group 3	R	R	S	28
Group 4	R	R	R	27

Short read whole genome sequencing is performed on these isolates using Illumina NovaSeq6000 to determine their multi locus sequence type and estimate the AMR genes' copy number through their mapping depth. A t-Distributed Stochastic Neighbor Embedding (t-SNE) clustering method was employed with CNV data as an input to determine if any isolates clustered based on their respective AMR gene content and CNV data.

Acknowledgements

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Figure 1: Multi locus sequence type prevalence in different groups

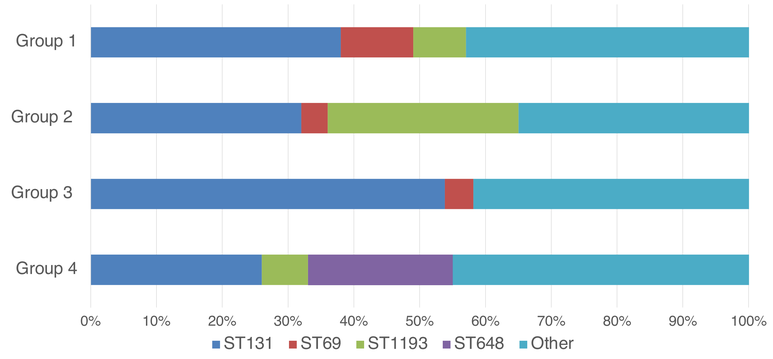


Figure 2: Maximum-likelihood Phylogeny in *E. coli* isolates

A maximum likelihood midpoint rooted tree showing the evolutionary relationships between isolates of various BL/BLI groupings. The tree was constructed using a multiple sequence alignment of the core genomes (genes present in 99% of isolates) and subsequent maximum likelihood model optimization of a neighbor-joining tree. Finally, the BL/BLI groupings were overlayed onto the tree tips.

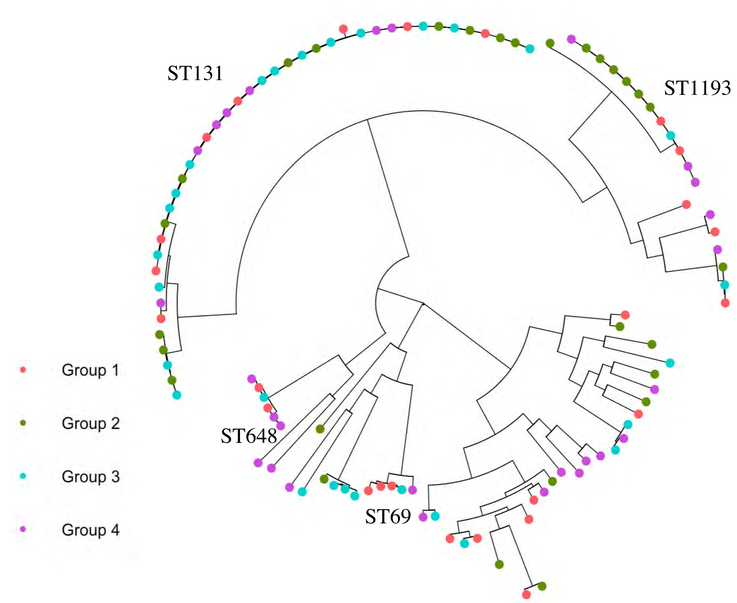
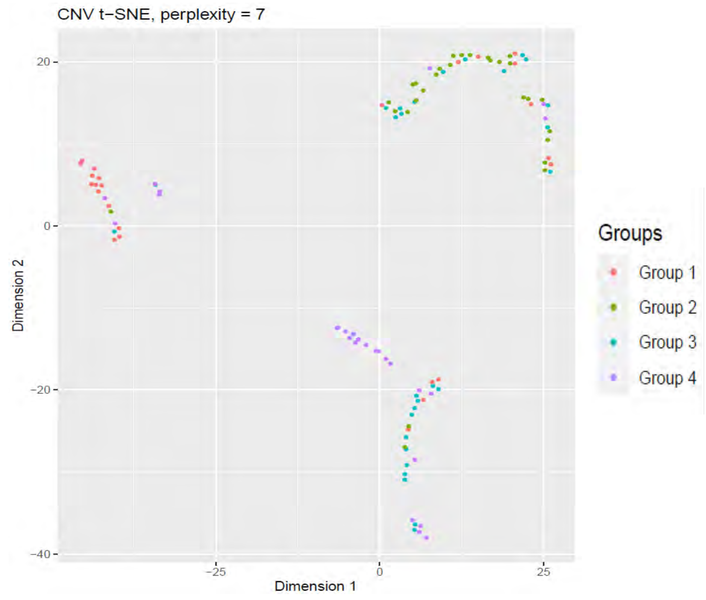


Table 2: Narrow Spectrum Beta-lactamase Characteristics Across ESRI Group Spectrum

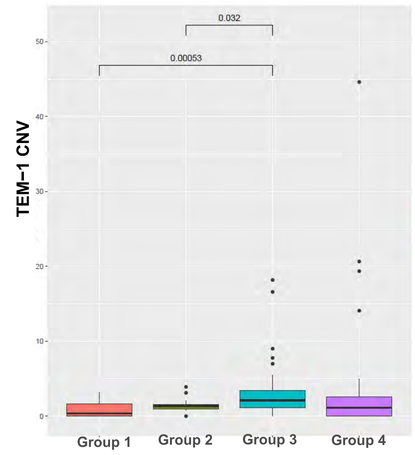
	Group 1 (n=26)	Group 2 (n=28)	Group 3 (n=28)	Group 4 (n=27)
bla_{TEM-1B} (n; %)	13 (50)	27 (96.4)	26 (92.9)	15 (55.6)
Copy Number (mean; sd)	1.8 (0.7)	1.5 (0.7)	3.7 (4.5)	8.2 (12.1)
bla_{OXA-1} (n; %)	0 (0)	0 (0)	1 (3.6)	17 (63.0)
Copy Number (mean; sd)	NA	NA	0.8 (NA)	5.2 (4.8)

Figure 3: t-SNE analysis based on genetic profiling and resistance patterns



To provide a qualitative description of the BL/BLI groupings associations with their AMR copy number variations (CNV), we conducted a t-distributed stochastic neighbor embedding (t-SNE). The transformation that produced the best approximation of population structure was with perplexity 7. Our t-SNE analysis shows that fully susceptible isolates (Group 1) and fully resistant isolates (Group 4) cluster independently.

Figure 4: Comparative boxplot on TEM-1 gene amplification



The boxplot comparison for TEM-1 shows a statistical significance in group 1/group 3 (0.00053) and group 2/group 3 (0.032). Also, OXA-1 is significantly more prevalent in group 4.

Conclusions/Further Discussions

- In the dataset examined, bla_{OXA-1} is overwhelmingly present in group four (17/27= 63%) with an elevated copy number. (5.2)
- Examination of the copy number estimates of the narrow spectrum beta-lactamase bla_{TEM-1} allows for us to distinguish between group 1/group 2 and group 3. (1.8/1.5 and 3.7)
- All ST648 present in our dataset are resistant to SAM/AMC/TZP (Group 4).
- Amongst the AMR genes scanned for clustering is helpful in predicting grouping for group 1 and group 4. In further study, we would like to expand on our current model by examining the regulators around the AMR genes in the hopes for creating a better model for distinguishing between group 2 and group 3.

Carbapenemase-producing Extraintestinal Pathogenic Escherichia coli from Argentina: Clonal Diversity and Predominance of Hyperepidemic Clones CC10 and CC131

Sanz MB[1†], De Belder D[1,2†], Mendieta JM[1], Faccone D[1,3], Poklepovich T[2], Lucero C[1], Rapoport M[1], Campos J[2], Tuduri E[1,2], Ojeda Saavedra M[4], Van der Ploeg C[5], Rogé A[5], "Carbapenemases-ExPEC Group", Pasteran F[1], Corso A[1], Rosato A[6,7§], Gomez SA[1,3§*]

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Abstract

Background. Extraintestinal pathogenic *Escherichia coli* (ExPEC) cause infections outside the intestine. Particular ExPEC clones, such as clonal complex (CC) or sequence type (ST)131, have been known to sequentially accumulate antimicrobial resistance that started with chromosomal mutations against fluoroquinolones and followed with the acquisition $bla_{\text{CTX-M-15}}$ and more recently carbapenemases.

Goal. Here, we aimed to investigate the distribution of global epidemic clones of carbapenemase-producing ExPEC from Argentina in representative clinical isolates recovered between July 2008 and March 2017.

Methods. Carbapenemase-producing E. coli (n=160) were referred to the Argentinean reference laboratory. Of these, 71 were selected as representative considering the year of isolation and geographical location. Phenotypic and microbiological assays were done to determine antimicrobial susceptibility testing and carbapenemase production with methods and interpretation according to CLSI. Whole genome sequencing was performed extracting DNA with QIACube DNAMini Kit. Library preparation was done with Nextera XT kit and the DNA was sequenced using Illumina's HiSeq2000 to generate 150 bp paired end reads. Reads were trimmed and assembled with Unicycler, annotated with Prokka and analyzed several tools including ARIBA, AMRfinder, SRST2, Tet-typer, among others. Phylogenetic analysis was performed with 9 reference strains using Roary, SNP-sites and RAxML to build the maximum likelihood tree under GTR model with gamma distribiution and 1000 bootstrap. MLST was determined submitting trimmed reads to Enterobase.

Results. Phenotypic and microbiological studies confirmed the presence of a carbapenemases confirmed as KPC-2 (n=52), NDM-1 (n=16), IMP-8 (n=2) and VIM-1 (n=1) producers. The isolates had been recovered from nine provinces and 46 health centers mainly from invasive infection sites like urine, blood, abdominal fluids, bones etc. and non invasive sites like rectal swabs. 76% of the isolates passed the virulence gene content criteria to be considered ExPEC although non-ExPEC isolates were also obtained from extraintestinal sites. Pan-genome phylogeny and clonal analysis showed great clonal diversity although the first phylogroup in abundance was phylogroup A, enclosing CC10 isolates, followed by phylogroup B2 with CC/ST131, mostly H30Rx, the subclone co-producing CTX-M-15. Phylogroup D, B1, C, F and E were also detected with fewer strains. CC10 was identified only in the area of the capital district, with the highest population density, while CC/ST131 was detected throughout the country. In addition, CC10 nucleated most metallo enzymes such as NDM-1. In addition, other relevant international clones were identified such as CC/ST38, CC155, CC14/ST1193 and CC23. Two isolates co-produced KPC-2 and OXA-163 or OXA-439, a point mutation variant of OXA-163, and three isolates co-produced MCR-1, among other resistance genes.

Conclusion. Herein, we described the molecular epidemiology of carbapenemase-producing ExPEC in Argentina and detected CC10 to be first in abundance instead of ST131. This work provides insight into the diversity of phylogroups, clones, subclones, serotypes, resistance and virulence genes of clinical carbapenemase-producing *E. coli* causing extraintestinal infections in Argentina.

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"Carbapenemase-producing Extraintestinal Pathogenic Escherichia coli from Argentina: Clonal Diversity and Predominance of Hyperepidemic Clones CC10 and CC131"

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Background

Extraintestinal pathogenic *Escherichia coli* (ExPEC) cause infections outside the intestine. Particular ExPEC clones, such as clonal complex (CC) or sequence type (ST)131, have been known to sequentially accumulate antimicrobial resistance that started with chromosomal mutations against fluoroquinolones and followed with the acquisition $bla_{\text{CTX-M-15}}$ and more recently carbapenemases (CBP).

Aim

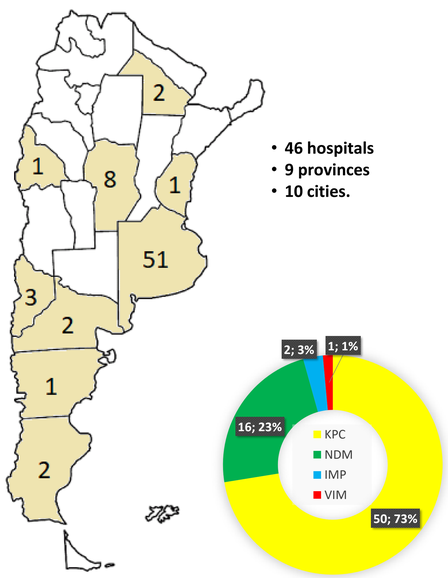
To investigate the distribution of global epidemic clones of carbapenemase-producing ExPEC (CP-ExPEC) from Argentina in representative clinical isolates recovered in Argentina between July 2008 and March 2017

Methods

- Carbapenemase-producing *E. coli* (n=160) were referred to the Argentinean reference laboratory INEI-ANLIS.
- Of these, 71 were selected as representative considering the year of isolation and geographical location.
- Phenotypic and microbiological assays were done to determine antimicrobial susceptibility testing and carbapenemase production with methods and interpretation according to CLSI.
- Whole genome sequencing was performed extracting DNA with QIACube DNAMini Kit. Library preparation was done with Nextera XT kit and the DNA was sequenced using Illumina's HiSeq2000 to generate 150 bp paired end reads.
- Reads were trimmed and assembled with Unicycler, annotated with Prokka and analyzed several tools including ARIBA, AMRfinder, SRST2, Tet-typer, among others.
- Phylogenetic analysis was performed with 9 reference strains using Roary, SNP-sites and RAxML to build the maximum likelihood tree under GTR model with gamma distribiution.

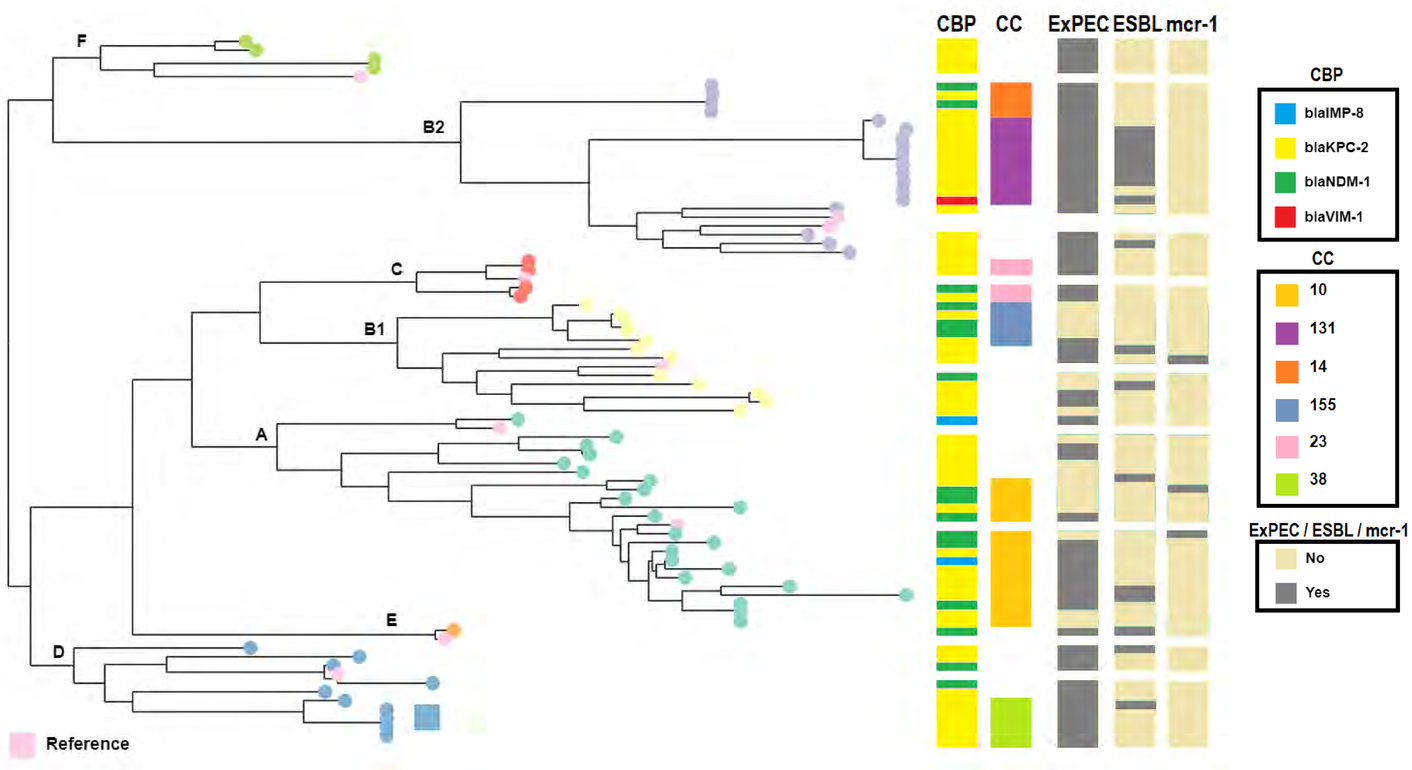
Results

Geographical Distribution of 71 CP-ExPEC



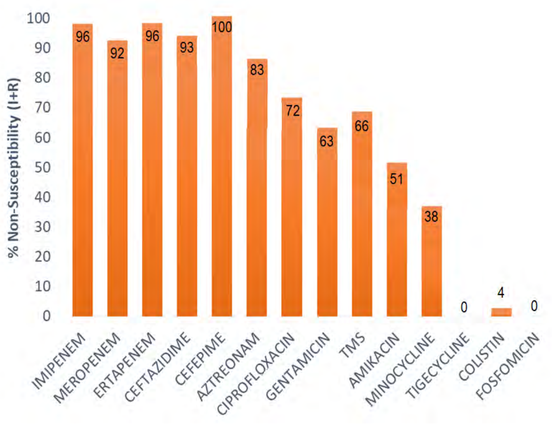
CP-ExPEC received at LNRRA

Phylogenetic tree of 71 clinical CP-ExPEC



- **Pan-genome phylogeny and clonal analysis showed great clonal diversity**
- **The first phylogroup in abundance was phylogroup A, enclosing CC10 isolates, followed by phylogroup B2 with CC/ST131, mostly H30Rx, the subclone co-producing CTX-M-15**
- **All clonal complexes clustered in monophyletic branches in each phylogroup. Relevant international clones were identified indicated in the figure with color code such as CC10, CC/ST131, CC/ST38, CC155, CC14/ST1193 and CC23.**
- **76% of the isolates passed the virulence gene content criteria to be considered ExPEC although non-ExPEC isolates were also obtained from extraintestinal sites.**
- **mcr-1 genes were detected in 3 isolates**
- **Two isolates co-produced KPC-2 and OXA-163 or OXA-439.**

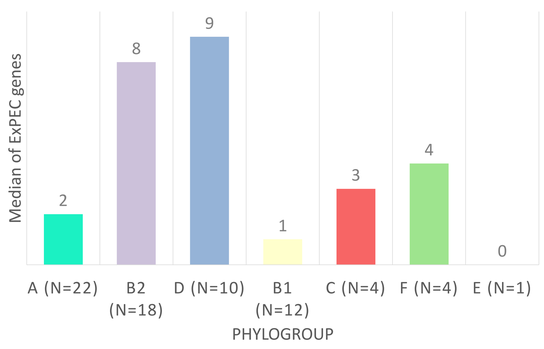
Antimicrobial resistance profile



Antimicrobial resistance is shown as % of non-susceptible considering intermediate + resistant categories (I+R).

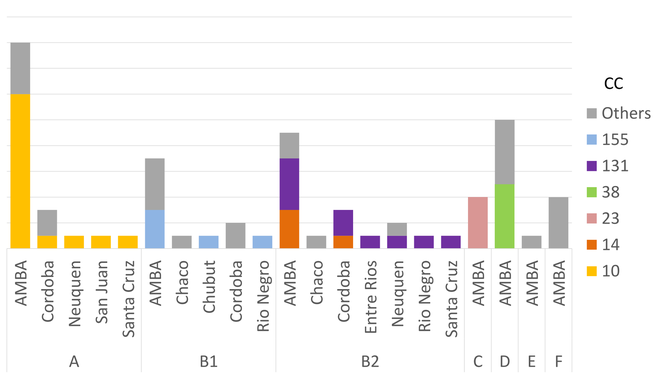
- **The most active drugs were minocycline, tigecycline, colistin and fosfomycin.**

Median of ExPEC genes per phylogroup



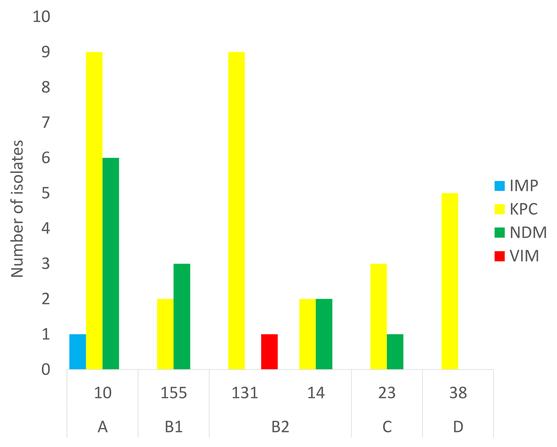
- **Phylogroups B2 and D have the highest number of virulence genes per strain as previously reported** (Sarowska, *et al.*, 2019).

Geographical distribution of Clonal Complexes and Phylogroups



- **.Phylogroups, A, B1 and B2 were detected throughout the country, while the majority of isolates in phylogroup D wer, also appears only in AMBA, like C, E and F. But these last 3 have a low n.**

CBP per phylogroup and CC



- ***bla*$_{\text{KPC-2}}$ was the major carbapenemase detected distributed across all phylogroups.**
- ***bla*$_{\text{NDM-1}}$ was mainly found in phylogroup A - CC10**
- ***bla*$_{\text{IMP}}$ was found in an isolate of phylogroup A and *bla*$_{\text{VIM}}$ in phylogroup B2.**

CONCLUSIONS:

- ✓ **Our findings show the phylogenetic distribution of Argentinian carbapenemase-producing *E. coli.***
- ✓ **We found a great genetic diversity distributed in 7 phylogroups, being the major phylogroups A, B1, B2 and D.**
- ✓ **CC10 was the first clone in abundance followed by CC/ST131**
- ✓ **76% of the isolates harbored the virulence genes to be considered as ExPEC. And phylogroups D and B2 had the highest score of virulence genes.**

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Comparison of Healthcare Resource Utilization (HRU) Among Adult Patients Treated with Omadacycline (OMC) for Acute Bacterial Skin and Skin Structure Infections (ABSSSI) or Community-Acquired Bacterial Pneumonia (CABP) in the 30 Days Pre- and Post-OMC Prescription (Rx)

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Background: Thirty-day outcomes are an important quality metric for both private and public payers. This retrospective study compared healthcare resource utilization (HCRU) among adult patients treated with OMC for ABSSSI or CABP, in the 30 days before versus after prescription of OMC.

Hypothesis/Goals: To assess 30-day real-world outcomes associated with omadacycline (OMC) for the treatment of adults with acute bacterial skin and skin structure infections (ABSSSI) or community-acquired bacterial pneumonia (CABP).

Methods: The pre/post study design was selected to assess how 30-day HCRU changed after OMC prescription (proxy for treatment response). Patients who received ≥1 OMC outpatient prescription were identified from a large US claims database (Oct 2018 to Sep 2020). Patients were classified as ABSSSI or CABP cohort based on presence of ICD-10 code near (−90 to +30 days) OMC prescription. Patients were classified as "complicated" if any of the following infections were identified in the pre-period (−90 to +5 days): ABSSSI: osteomyelitis (OST), sepsis/bacteremia (S/B), endocarditis, implant, necrotizing fasciitis, meningitis; CABP: severe pneumonia, lung abscess, S/B, endocarditis, meningitis. Risk of inpatient admissions, emergency department (ED) visits, and outpatient visits were compared between 30 days before versus 30 days after OMC prescription.

Results: During the study period, 258 OMC outpatient prescriptions (189 ABSSSI, 69 CABP) met the inclusion criteria. Of the 189 ABSSSI patients, 83 were classed as complicated. The most common ABSSSI complications were OST (53%), S/B (33%), and implant infection (21%). Among the 69 CABP patients, 20 were classed as complicated. Most common CABP complications were S/B (80%) and severe pneumonia (25%). Among complicated ABSSSI patients, hospital admissions decreased by 38% (41% vs 25%; $p<0.05$) while ED and outpatient visits were similar. Among non-complicated ABSSSI patients, hospital admissions decreased by 61% (17% vs 7%; $p<0.05$); ED visits decreased by 88% (16% vs 2%; $p<0.01$), and outpatient visits were similar. Among complicated CABP patients, hospital admissions decreased by 75% (80% vs 20%; $p<0.01$); ED visits decreased by 100% (40% vs 0%; $p<0.001$), and outpatient visits were similar. Among non-complicated CABP patients, hospital admissions decreased by 75% (33% vs 8%; $p<0.01$); ED and outpatient visits were similar.

Conclusions: This study provided the first real-world characterization of patients treated with OMC for ABSSSI or CABP. Patients who received OMC had lower HCRU in the 30 days after OMC prescription, relative to the 30 days before.

Acknowledgments: This study was funded by Paratek Pharmaceuticals, Inc.

Disclosures: TPL is a consultant and speaker for Paratek Pharmaceuticals, Inc. KG, MR, HH, SS, and GB are employees and shareholders of Paratek Pharmaceuticals, Inc. FM, EG, and DY are employees of Analysis Group, which received consulting fees from Paratek Pharmaceuticals, Inc.

Comparison of Healthcare Resource Utilization Among Adult Patients Treated with Omadacycline for ABSSSI or CABP in the 30 Day Pre- and Post-Omadacycline Prescription

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Background

Community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI) continue to be a substantial cause of morbidity and mortality in the United States[1–3]

Ambulatory visits for CABP and ABSSSI are ~4.5 million and 8.4 million per year in the United States respectively[4–6]

Annual medical costs linked to pneumonia exceed $17 billion, and among the Medicare population, the burden of CABP costs was estimated to be $13 billion in 2008.[7] Limited information is available on the burden of ABSSSI

Omadacycline, a first in class oral and intravenous (IV) once-daily antibiotic, recently approved for the treatment of adult patients with CABP and ABSSSI, with activity against key pathogens, S. pneumoniae and S. aureus including drug resistant strains

Methods

US claims data from the PatientSource® database, supplied by Source Healthcare Analytics, LLC, a Symphony Health Solutions Corporation covering ~274 million patients were used for this study, including data from October 2018 to September 2020

A pre-post study design was selected to assess how patient outcomes changed pre vs. post-omadacycline prescription

Patients treated with omadacycline (index date: prescription date) for ABSSSI or CABP who had 3 months of data available prior to the index date (baseline period) and 1 month of data following the index date (study period) were included in the study

Patients were classified as complicated and uncomplicated based on infection history

Statistical analysis

- Baseline characteristics and treatment characteristics were compared using ANOVA tests for continuous variables and Chi-squared tests for categorical variables
- Healthcare resource use (HRU), including all-cause and disease-specific visits, were assessed and compared between the pre- and post-periods. P-values were generated using generalized estimating equation (GEE) regression with robust sandwich estimators to account for within-patient correlation

Table 1. Baseline characteristics among ABSSSI patients in the 90-day pre-omadacycline prescription period

	Complicated ABSSSI (N = 83)	Non-complicated ABSSSI (N = 106)
Demographics		
Age on index date in years, mean ± SD	58.11 ± 13.46	57.81 ± 15.98
Female, n (%)	37 (44.6)	63 (59.4)
Region, n (%)		
South	37 (44.6)	49 (46.2)
Midwest	21 (25.3)	28 (26.4)
Northeast	15 (18.1)	20 (18.9)
West	10 (12.0)	8 (7.6)
Unknown	0 (0.0)	1 (0.9)
Clinical characteristics		
CCI	1.64 ± 1.68	1.05 ± 1.44
Comorbidities of interest, n (%)		
Osteomyelitis	44 (53.0)	0 (0.0)
Diabetes	43 (51.8)	35 (33.0)
Septicemia/sepsis/bacteremia	27 (32.5)	0 (0.0)
Peripheral vascular disease	27 (32.5)	10 (9.4)
Renal diseases	21 (25.3)	18 (17.0)
Chronic obstructive pulmonary disease	17 (20.5)	20 (18.9)
Implant/graft/prosthetic joint infection	17 (20.5)	0 (0.0)
Congestive heart failure	14 (16.9)	11 (10.4)
Urinary tract infection	10 (12.0)	3 (2.8)
Surgical site infection	7 (8.4)	7 (6.6)
At least one non-Nuzyra antibiotic in the baseline period	68 (81.9)	82 (77.4)

Table 2. Baseline characteristics among CABP patients in the 90-day pre-omadacycline prescription period

	Complicated CABP (N = 20)	Non-complicated CABP (N = 49)
Demographics		
Age on index date in years, mean ±SD	64.30 ± 17.34	63.18 ± 14.61
Female, n (%)	11 (55.0)	30 (61.2)
Region, n (%)		
South	18 (90.0)	38 (77.5)
Midwest	1 (5.0)	4 (8.2)
Northeast	0 (0.0)	4 (8.2)
West	1 (5.0)	3 (6.1)
Unknown	0 (0.0)	0 (0.0)
Clinical characteristics		
CCI	2.30 ± 1.98	1.63 ± 1.74
Comorbidities of interest, n (%)		
Septicemia/sepsis/bacteremia	16 (80.0)	0 (0.0)
Chronic obstructive pulmonary disease	11 (55.0)	24 (49.0)
Renal diseases	8 (40.0)	9 (18.4)
Congestive heart failure	6 (30.0)	8 (16.3)
Complicated pneumonia	5 (25.0)	0 (0.0)
Diabetes	5 (25.0)	6 (12.2)
Bacterial pneumonia (other than CABP)	3 (15.0)	7 (14.3)
Rheumatic diseases	3 (15.0)	3 (6.1)
Peripheral vascular disease	2 (10.0)	5 (10.2)
Urinary tract infection	2 (10.0)	2 (4.1)
Any malignancy	2 (10.0)	3 (6.1)
Mild liver disease	1 (5.0)	3 (6.1)
Clostridioides difficile infection	1 (5.0)	3 (6.1)
At least one non-Nuzyra antibiotic in the baseline period	20 (100.0)	43 (87.8)

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HRU reductions after treatment with omadacycline for both ABSSSI and CABP

Objectives

To address the evidence data gap around real-world use of omadacycline, this study had the following objectives:

- To understand patient and treatment characteristics among those with an omadacycline prescription for CABP and ABSSSI
- To assess healthcare resource use associated with the use of omadacycline for CABP and ABSSSI in the pre- and post-omadacycline prescription period

Conclusions

This study provided the first real world characterization of patients treated with omadacycline for ABSSSI or CABP, including patients with high comorbidity burden and extensive use of antibiotics

After receiving omadacycline, patients had lower all-cause and disease-specific HRU, including inpatient visits, in the 30-day post-omadacycline prescription period relative to the 30-day pre-omadacycline prescription period

HRU reductions after treatment with omadacycline were consistent among complicated and non-complicated patients for both ABSSSI and CABP

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Results

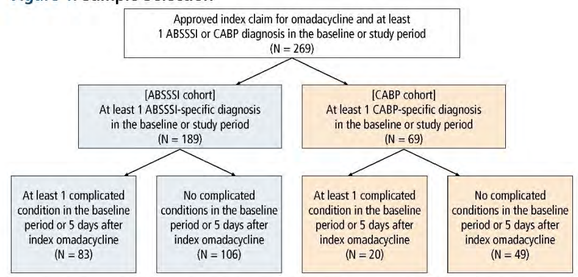
The study population selection is shown in **Figure 1**

A high proportion of patients in both the ABSSSI and CABP cohorts experienced prior bacterial infections in the baseline period and patients had a high comorbidity burden (**Tables 1 and 2**)

In the baseline period, most patients were treated with at least one prior antibiotic class

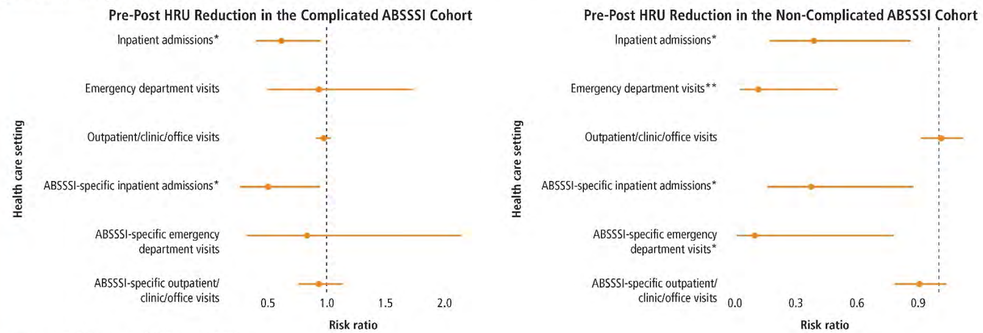
All-cause and disease-specific HRU showed significant reduction in the 30-day post-omadacycline prescription vs. 30-day pre-omadacycline prescription (**Figures 2 and 3**)

Figure 1. Sample Selection



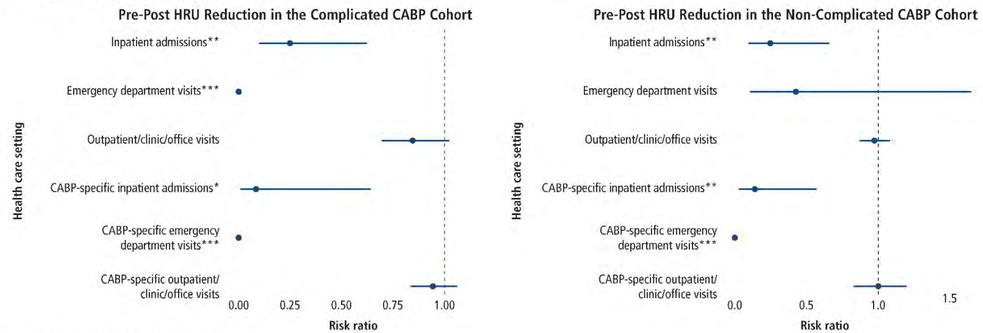
(Definition of complicated cohorts: ABSSSI: osteomyelitis, bone/joint infection, sepsis/bacteremia, endocarditis, implant infection, necrotizing fasciitis, meningitis; CABP: severe pneumonia, lung abscess, sepsis/bacteremia, endocarditis, meningitis).

Figures 2a and 2b. Comparison of HRU in the 30-day pre-omadacycline prescription vs. 30-day post-omadacycline prescription period among ABSSSI patients



*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001

Figures 3a and 3b. Comparison of HRU in the 30-day pre-omadacycline prescription vs. 30-day post-omadacycline prescription period among CABP patients



*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001

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Delafloxacin in Clinical Practice: A Single Center Study

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Background
The newest fluoroquinolone, delafloxacin (DLX) provides broad-spectrum coverage, improved side effect profile relative to older agents, and excellent potency. The drug has received US Food and Drug Administration (FDA) indications for the treatment of acute bacterial skin infections and community-acquired pneumonia.

Hypothesis/Goals
DLX may be useful in the treatment of other infections given the longstanding versatility of other fluoroquinolones. Currently no published data are available to support this hypothesis, however. Our goal was to analyze recent DLX use at our academic medical center and provide insight into other potential clinical niches for this antibiotic.

Methods
This is a retrospective review of adult patients treated with DLX at The University of Texas Medical Branch, Galveston, TX from January 1, 2018 to February 1, 2020 using pre-existing electronic medical records. Simple statistics were calculated using Microsoft Excel.

Results
Six patients were prescribed DLX (median age 59 years, 50% female, 100% outpatient) with a median treatment duration of seven days. Prescriptions were initiated by infectious diseases specialists (2/6, 33.3%), emergency medicine physicians (2/6, 33.3%), urologists (1/6, 16.7%), and ophthalmologists (1/6, 16.7%). The most common conditions treated were prosthetic joint infections (PJI) and acute skin and soft tissue infections (each n = 2). Both PJIs were caused by multi-drug-resistant *Staphylococcus epidermidis*. Off-label utilization was high (4/6, 66.7%). All patients (6/6, 100%) experienced clinical improvement and/or cure with delafloxacin. There were zero reported adverse events.

Conclusions
A new antibiotic with valuable characteristics, DLX treatment was safe and successful in this retrospective analysis, including with several off-label indications. Real-world clinical data with delafloxacin are currently scant. Prospective data would be useful for identifying future clinical niches for this new fluoroquinolone.

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Delafloxacin in Clinical Practice: A Single Center Study

- J. Patrik Hornak, MD - David Reynoso, MD, PhD -
The University of Texas Medical Branch, Galveston, TX

Introduction

- One of the newest fluoroquinolones, delafloxacin (DLX) offers broad-spectrum activity, relatively favorable side effect profile, and excellent potency.
- DLX's antimicrobial spectrum includes *Pseudomonas aeruginosa*, anaerobes, MRSA, among other flora.
- The drug is currently approved for acute bacterial skin and skin structure infections (ABSSTIs) and community-acquired pneumonia (CAP).
- Fluoroquinolones are frequently used for a wide variety of infections beside ABSSTI and CAP. DLX may be useful in these situations.
- Currently, no real-world data are available to further explore DLX's potential role(s) in treating other infectious diseases.

Methods & Patient Selection

Single-center, retrospective review at UTMB Health from Jan 1, 2018 to Feb 1, 2020 of adult patients prescribed DLX for any indication. Statistics performed with Microsoft Excel.

Items of interest
- Disease / syndrome
- Patient characteristics
- Prior antimicrobials & isolate susceptibilities
- Prescribing clinician type (i.e. specialty)
- DLX monotherapy vs combination strategy

Outcomes
- Clinical improvement and/or resolution
- Relapse
- Replacement by alternative antimicrobial
- Hospital readmission within 30 days of DLX receipt
- Adverse effects

Patient factors & characteristics
- Age
- Sex
- Ethnicity
- Comorbid conditions
- Inpatient vs outpatient setting

Patient Population

Table 1, Study Population	n = 5
Age, years, median (range)	59 (30 - 94)
Female sex	2 (40%)
Non-white ethnicity	1 (20%)
Comorbid condition(s) present	3 (60%)
>Cardiovascular disease	2 (40%)
>Diabetes	1 (20%)
>Chronic kidney disease	0 (0%)
>Chronic liver disease	0 (0%)
>Immunocompromised state	0 (0%)
>Active or prior cancer	0 (0%)
>Stroke	1 (20%)

Treatment Details

Table 2, Clinical Features	
Outpatient treatment	5 / 5 (100%)
Prescriber specialty	
>Infectious disease	2 / 5 (40%)
>Emergency medicine	2 / 5 (40%)
>Ophthalmology	1 / 5 (20%)
Condition(s) treated	
>ABSSTI (cellulitis)	2 / 5 (40%)
>Prosthetic joint infection (PJI)	2 / 5 (40%)
>Ruptured globe antimicrobial prophylaxis	1 / 5 (20%)
Treatment details	
>DLX duration, days, median (range)	7 (7 - 115)
>Oral route of administration	5 / 5 (100%)
>Standard dose, 450mg q12h	5 / 5 (100%)
>Monotherapy	5 / 5 (100%)
Other antibiotic(s) used before DLX	3 / 5 (60%)

Clinical Results

Table 3, Outcomes	
Clinical outcome	
>Documented cure or improvement	4 / 5 (80%)
>Documented failure	0 / 5 (0%)
>Unknown	1 / 5 (20%)
>Hospital readmission within 30 days of DLX treatment	0 / 5 (0%)
>Therapeutic switch to an alternative antibiotic	1 / 5 (20%)
Adverse effects	
>Documented side effects attributed to DLX	0 / 5 (0%)

Discussion

Highlights from our Experience
- DLX was successful in treating numerous conditions, including several off-label indications.
- DLX treatment was safe and no apparent side effects were documented or clinically apparent.
- Two cases of difficult-to-treat PJIs demonstrated significant clinical improvement on DLX therapy.
- DLX is being prescribed by infectious disease (ID) and non-ID physicians.

Study limitations
- Small study, single centered, case series
- One case lost-to-follow up
- Insufficient design to propose non-inferiority or superiority

Potential Role(s) in Therapy & Remaining Questions
- *Neisseria gonorrheae* infections
- Atypical mycobacterial infections
- Prosthetic joint / hardware infections
- GI / GU infections (incl. *Helicobacter pylori* gastritis)
- Resistance development? Cost?
- **Larger, prospective studies will be very welcomed!**

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Seeing the Invisible: Deciphering Spectrophotometry-Based Time-Kill Measurements to Guide the Design of Antibiotic Dosing Regimens

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Abstract

Background: Personalized treatment of challenging bacterial infections would typically require laborious and time-consuming time kill experiments that entail standard plating methods. An efficient alternative would be the use of optical density (OD) measurements through a spectrophotometer. Unfortunately, such an instrument provides counts of both live and dead cells. In prior work, we demonstrated how mathematical modeling can use OD measurements to estimate the sizes of live and dead bacterial populations over time. This information can be used to predict the efficacy of corresponding dosing regimens under realistic pharmacokinetics. In this work, we study the feasibility of this approach, using in vitro simulation with a hollow fiber system.

Goals: Confirm that mathematical modeling using time-kill data produced by an OD instrument can be used to correctly predict the efficacy of corresponding dosing regimens for Ceftazidime (following given pharmacokinetics) on *Acinetobacter baumanii.*

Methods: Experimental: OD measurements of both live and dead cells combined for antibiotic concentrations $C = 0, 1, 4, 16, 64, 256$ (mg/L), are collected every 5 mins over a time period of 20 hours. Computational: OD measurements are used as inputs into a mathematical model and parameter estimation generates values of the kill rate of the most resistant bacteria sub-population at each C. That information is then used to design dosing regimens with high and low probability of eradication of the entire bacterial population. These dosing regimens are tested in a hollow fiber system.

Results: The dosing regimens tested in the hollow fiber system performed as anticipated.

Conclusions: The proposed modeling approach can make use of otherwise unusable OD time kill measurements for the design of effective dosing regimens under realistic antibiotic pharmacokinetics. The overall approach can be highly automated, and can be extended to combinations of antibiotics against multi-drug resistant bacteria.

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Deciphering Longitudinal Optical Measurements to Guide the Design of Antibiotic Dosing Regimens: A Model-Based Approach

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Overview

- Issue: Rapidly determine **antibiotics** and **dosing regimens** for treatment of **challenging bacterial infections**
- State of the art: **MIC** from time-kill experiments using (a) **plating**, (b) **optical-density (OD)** measurements
- Limitations: Not very informative: (a) **few** data points, (b) both **live+dead** bacterial cell counts
- Proposed solution: Use **math modeling** with **OD data** to **infer live** from **live+dead** cell counts over time and quantify antibiotic killing action. **Advantage**: Informative, efficient
- Case study: Use modeling on time-kill **OD data** to **predict** antibiotic effect under realistic PK and **validate** in a **hollow-fiber infection model**

Objective

- Develop an integrated approach towards **rapidly** and **reliably** making decisions on **personalized** dosing of **antibiotics** to combat resistant bacteria

Materials

- Antibiotic: **Ceftazidime** (CAZ)
- Microorganism: ***Acinetobacter baumannii*** (AB), ATCC BAA747

Methods

- Data Production: Perform automated time-kill experiments using an OD instrument for CAZ at $\mathbf{C = 0, 1, 4, 16, 64, 256}$ (mg/L) in triplicates
- Data Analysis: Use a mathematical model to fit the data and derive informative parameters on the killing effect of CAZ on AB at each antibiotic concentration C.
- Model Statistical Analysis: Use the fitted model to predict killing effect of CAZ periodically injected (half-life of exponential decay: 2.5 h) at various peak concentrations. Determine dose and dosing period that would eradicate the bacterial population.
- Experimental Validation of Results: Use of a hollow-fiber infection model to confirm predictions made using the fitted model.

Results

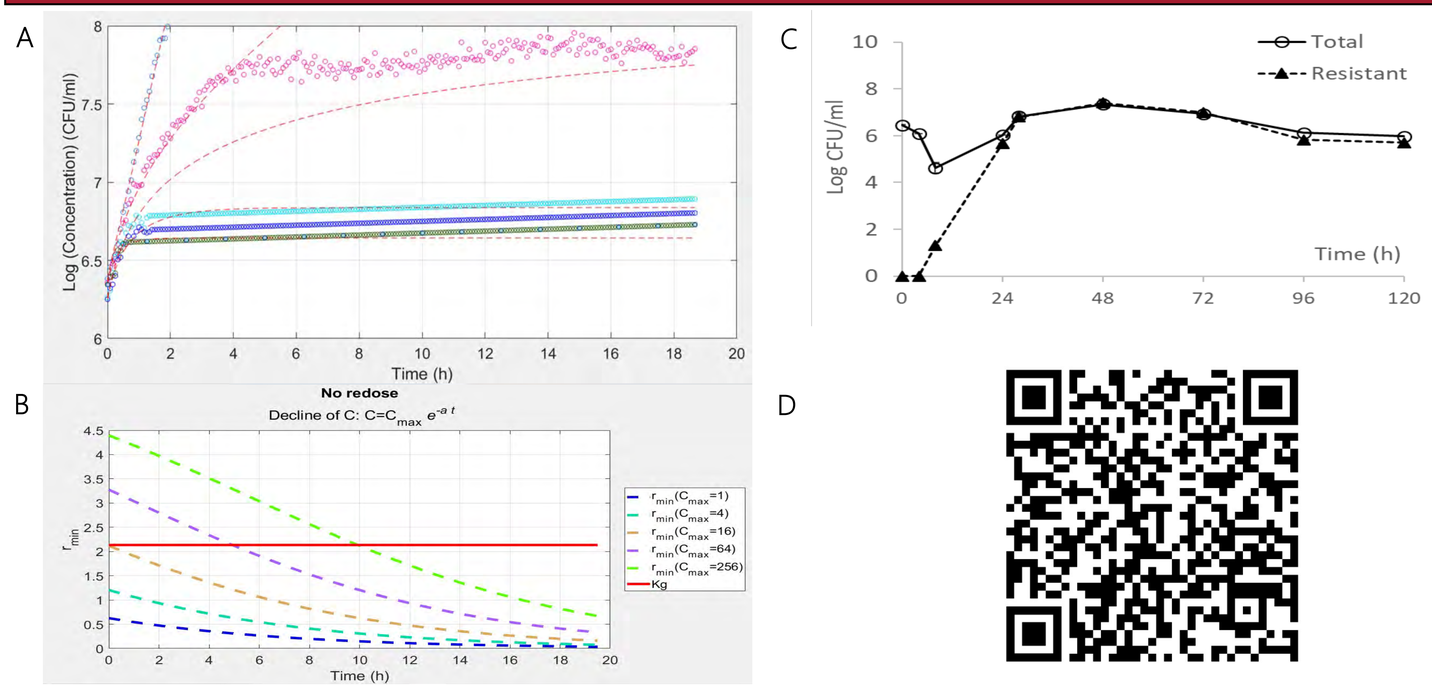

Fig. A: *Fit of TG and TK models to different lengths of experimental data generated by the OD instrument for a bacterial population of AB exposed to CAZ at a number of time-invariant $C = 0, 1, 4, 16, 64, 256\ mg/L$ for 20 hours. Data Restrictions: a) Max y-axis $C = 8\ log$ b) Elimination of decreasing total bacterial population*
Fig B: *Killing rate for each CAZ concentration under realistic PK of $2.5h$ half-life. The red line indicates the natural bacterial grown under which there is no more killing*
Fig C: *HFIM experiment, bacterial response to ceftazidime dosing regimen $2.5\ g$ (target $C_{\max} = 150\ mg/L$) administered every $8\ h$. Data shown as mean +/- SD*
Fig. D: ***Take a picture to access relevant model equations***

Discussion

- Predictions of Experimental Results are satisfactory
- Future work/ Current Issues: Repeat case-study with an Antibiotic that allows complete elimination of the Microorganism. High error in the derived parameters used in the prediction and further bibliography investigation is needed.

Antibiotic Interactions with Bacteriophage in Pseudomonas Biofilms and Planktonic Environments

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Background: *Pseudomonas aeruginosa* is a ubiquitous bacterial pathogen involved in a variety of infections from ventilator acquired pneumonias to diabetic feet. It can be difficult to treat owing to biofilm formation and both intrinsic and acquired resistances to antibiotics. Bacteriophages, the most diverse infectious agent on Earth, present a possible solution to this problem.

Hypothesis/Goals: We set out to test the hypothesis that varying combinations of bacteriophage and antibiotics will have different abilities to treat Pseudomonas in the biofilm environment

Methods: We characterised a novel phage, ΦJB10, using standard techniques, including genome sequencing and electron microscopy. We measured planktonic growth curves of Pseudomonas using OD600 while treating with varying combinations of antibiotics and ΦJB10. We measured the effects of these combinations on biofilms measuring the biomass with crystal violet and the number of metabolically active cells using MTT.

Results: Our data on the basic parameters of ΦJB10 revealed a very fast latent period of <10 minutes. In the planktonic form, ΦJB10 resulted in rapid clearance of Pseudomonas, however regrowth of phage resistant cells was rapid. We found that this could be suppressed by supplementation with anti-Pseudomonal antibiotics. Biofilm experiments showed that cefepime performed poorly on its own against biofilms, but synergy was found with ΦJB10. We also found that amikacin and meropenem acted as antagonists dynamically, but these effects had no impact on biofilm clearance at 24 hours. Further, we found that adjunctive ΦJB10 worked to reduce the MBEC50 to ciprofloxacin, amikacin and cefepime.

Conclusion: ΦJB10 acts with potent synergy with cefepime against both planktonic and Pseudomonas biofilms. Adjunctive phage also reduced MBEC50 to more favourable ranges for several antibiotics. These together show that appropriately selected combinations of phage and antibiotics can aid in clearing Pseudomonas in both planktonic and biofilm states.

Acknowledgements: We extend our thanks to the entire Maresso lab and TAILOR Labs for their valued advice. This work was supported by NIH training grant T32AI055413-17 as well as funding from the Kleberg Foundation to AWM.

Antibiotic Interactions with Bacteriophage in Pseudomonas Biofilms and Planktonic Environments

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Department of Medicine[1] and Department of Molecular Virology and Microbiology[2],
Baylor College of Medicine

Baylor College of Medicine

Introduction

Multidrug resistant bacteria (MDR) have been identified as a critical threat by the WHO and amongst these Pseudomonas aeruginosa (PsA) is a common isolate that is frequently resistant to antimicrobials[1,2]. While there is a new generation of antibiotics, resistant isolates have already developed[3]. Bacteriophages, viruses that infect and kill bacteria, are a possible solution. They were discovered in the early 20th century and used extensively in the former Soviet Union while the West used antibiotics[4]. Now with the frequency of antibiotic resistance, there is renewed interest in phage therapy.

Bacteriophages are extremely numerous, with 10^{31} infectious particles on Earth. They are typically non enveloped, dsDNA viruses that have specific tropisms to different bacteria and are frequently specific to individual strains. While bacteriophages have been successful in case reports and disparate case series, there has not yet been success in any larger clinical trial[5,6].

Pseudomonas is notorious for chronic infections and can be seen in hardware infections, diabetic feet and cystic fibrosis[7]. In these situations, the presence of biofilm is usually prominent. Against these biofilms antibiotics are less effective but some bacteriophages are known to have excellent activity against biofilms. Here, we investigated the interactions between bacteriophages and anti-Pseudomonal antibiotics in both planktonic and biofilm forms.

Methods

Strains: Phage ΦJB10 was discovered and purified from fresh water obtained from Clear Creek in League City, TX. *Pseudomonas aeruginosa* PAO1 is a common laboratory strain and was cultured with usual techniques.

Phage Discovery and Purification: Fresh water samples were obtained and mixed with PAO1 in 0.8% top agar, streaked for isolation, expanded in PAO1 in LB media and precipitated using PEG/NaCl without use of chloroform and purified by CsCl gradient. The resulted phage band was dialysed against 10mM Tris pH 8.0, 100mM NaCl, 10mM $MgSO_4$.

Antibiograms: Gradients of phage and antibiotics were set up in 96 well plate format with total volume of 100uL in each well and mixed with 100uL PAO1 OD600=1.0. OD600 was monitored in a Synergy HTX plate reader.

Biofilms Experiments: PAO1 are seeded in a tissue culture treated 48 well plate, cultured overnight and then treated with combinations of phage or antibiotic as indicated. Biofilms were evaluated with either crystal violet or MTT staining. Statistical comparisions were done by 1 way ANOVA.

Results

ΦJB10 is a member of Podoviridae with a rapid life cycle

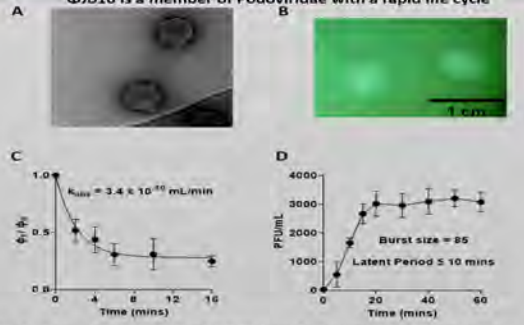

Fig 1: We isolated and purified ΦJB10 from fresh water from Clear Creek TX. Electron microscopy with negative staining (A) revealed a *Podovirus* and sequencing confirmed this. It formed plaques with a prominent halo on PAO1 top agar (B). Life cycle studies reveal adsorption constant within the normal range (C), but a very short latent period, allowing rapid amplification in culture (D).

Results

Synography reveals phage-antibiotic interactions in planktonic form

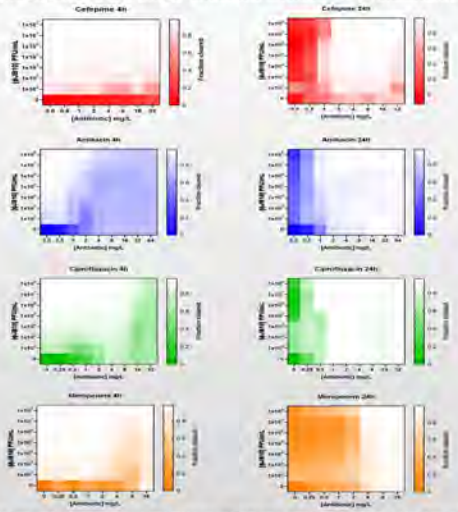

Fig 2: We mixed various concentrations of phage and antibiotic with log-phase PAO1 Pseudomonas and monitored clearance of the cultures and here we show the fraction cleared in a heat map format. At early time points (4 hours) phage provides effective clearance but amikacin, ciprofloxacin and meropenem show concentration dependent antagonism with ΦJB10. By 24 hours: 1) Antagonism is no longer seen, 2) cefepime shows synergy with the phage, 3) presence of any antibiotic class is sufficient to stop regrowth of the Pseudomonas.

ΦJB10 rapidly clears biofilm and shows class dependent interaction with antibiotics

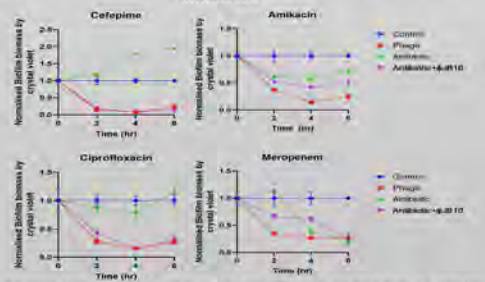

Fig 3: A) Cefepime 8mg/mL, B) Amikacin (16 mg/mL), C) Ciprofloxacin (0.5mg/mL), D) Meropenem (8 mg/mL). Mean is plotted, and error bars represent SEM of 6 biological repeats, assayed in triplicate.

Phage treatment resulted in rapid eradication of >80% of the biofilm within 4 hours. Amikacin and meropenem seemed to act as antagonists to this effect. No dynamic interactions were seen with ΦJB10 and cefepime or ciprofloxacin.

Results

Durable biofilm clearance requires presence of any antibiotic, but synergy is seen between ΦJB10 and cefepime

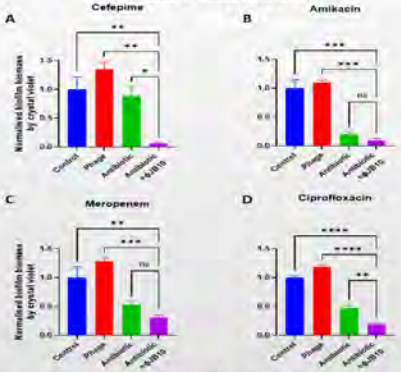

Fig 4: A) Cefepime 8mg/mL, B) Amikacin (16 mg/mL), C) Ciprofloxacin (0.5mg/mL), D) Meropenem (8 mg/mL). Mean is plotted, and error bars represent SEM of 6 biological repeats, assayed in triplicate. ns > 0.05, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001

Here, we tested whether phage or phage-antibiotic combinations could provide durable clearance of the Pseudomonas biofilm at 24 hours. In the phage only treatment group, biofilms showed complete regrowth beyond even the LB only control. Antibiotics showed class dependent ability to eradicate the biofilm. Cefepime was the least active while amikacin was the most active. In all situations addition of phage to the antibiotic resulted in better clearance but this was statistically significant for only cefepime and ciprofloxacin.

MTT Assay confirms class dependent interactions between phage and antibiotics in biofilm environments

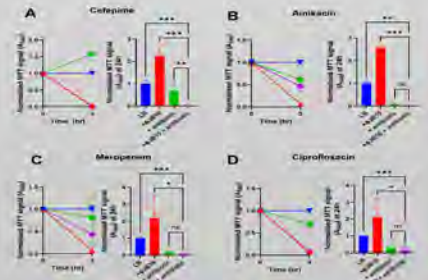

Fig 5: A) Cefepime 8mg/mL, B) Amikacin (16 mg/mL), C) Ciprofloxacin (0.5mg/mL), D) Meropenem (8 mg/mL). Mean is plotted, and error bars represent SEM of 6 biological repeats, assayed in triplicate. ns > 0.05, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001

We repeated similar biofilm experiments, treating with phage, antibiotic or phage antibiotic combination however this time we used MTT to measure only metabolically active cells as crystal violet will stain non living biomass. We found very similar results, validating our earlier ideas

Results

Adjunctive bacteriophage can result in lower MBEC50 to antibiotics

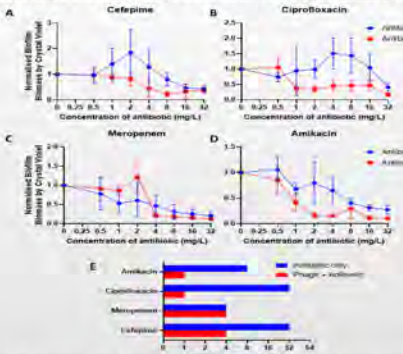

Fig 6: A) Cefepime, B) Amikacin, C) Ciprofloxacin, D) Meropenem ± ΦJB10 at 10^8 PFU/mL. Data points represent mean and error bars SD of 4 independent biological experiments. E) Median MBEC50 extrapolated from the independent experiments.

Addition of bacteriophage to antibiotics at the indicated concentration (A-D) resulted in reductions to the median MBEC50 dependent upon the class of antibiotic (E). These changes are large enough they would have the potential to change clinical outcomes.

Conclusion

We demonstrate:

1) the basic phage characteristics of ΦJB10, with a remarkably short latent period
2) Dynamic antagonism between ΦJB10 and amikacin, ciprofloxacin and meropenem in planktonic experiments
3) Dynamic antagonism between ΦJB10 and both amikacin and meropenem in biofilm experiments
4) Synergy between cefepime and ΦJB10 in planktonic and biofilm forms measured by crystal violet for biomass and MTT for metabolic activity
5) Possible reduction in MBEC50 by ΦJB10 for amikacin, cefepime and ciprofloxacin

Further investigation

We will extend our investigations to see if these patterns of interactions hold for more than only the ΦJB10 used in these experiments. Generalisation of phage-antibiotic interactions will allow easier formulation of effective phage cocktails with favourable interactions with concurrent antibiotics.

Acknowledgements and References

I would like to extend my thanks to all members of the Maresso Lab, especially Carmen Gu Liu, for invaluable assistance and counsel as well as to TAILΦR Labs for their technical advice and experience. This work was supported by NIH training grant T32AI055413-17 as well as fundrig from the Kleberg Foundation to AWM.

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

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

Methods: We generated *liaF* mutants in *E. faecalis* OG1RF and OG117 (derivate of OG1RF) harboring: ***i)*** four stop codons leading to premature truncation of the protein (OG117*liaF**$_{11\text{-}14}$), and *ii)* an allele coding for deletion of isoleucine in position 177 (OG1RF*liaF*Δ_{177}). In addition, we complemented in *cis* the OG117*liaF*11-14* mutant using the CRISPR-Cas9 system with wild type *liaF*. We also complemented OG1RF*liaF*Δ_{177} *in trans* using the nisin induced vector pMSP3535. DAP MICs were performed using E-test. In addition, broth microdilution DAP MIC were made in presence/absence of the N-terminal. We evaluated cell membrane anionic phospholipid (AP) microdomain localization using 10-*N*-nonyl-acridine-orange (NAO) and determined activation of LiaFSR by evaluating surface exposure of LiaX by ELISA. Lastly, we used the bacterial adenylate cyclase two-hybrid system (BACTH) to study the protein-protein interaction between LiaF-LiaS, LiaFΔ_{177}-LiaS and LiaF-LiaR.

Results: The insertion of four stop codons 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, LiaF$_{\Delta 177}$ increased surface exposure of LiaX more than 8-fold and produced a redistribution of anionic phospholipid microdomains away from the septum without changes in the DAP MIC. Complementation *in trans* of with the *liaF* wild type allele in OG1RF*liaF*Δ_{177} resulted in partial restoration of the septum distribution. DAP susceptibility did not change in the OG1RF*liaF**$_{11\text{-}14}$ 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*$_{*11\text{-}14.}$ 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 interactions were 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.

Funding: NIH/NIAID R01AI134637, K08 AI113317

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

Diana Panesso[1-3], Sara I. Gomez-Villegas[1], Sandra L. Rincon S[3] Ayesha Khan[4-6], April Nguyen[1,7], Yousif Shamoo[8], Truc T. Tran[1-2], Cesar A. Arias, [1-2]

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Abstract

Background: Daptomycin (DAP) is a lipopeptide antibiotic used as a first-line agent for the treatment of vancomycin-resistant enterococcal infections. Resistance to DAP in enterococci is controlled by the LiaFSR three-component regulatory system. LiaS encodes for the histidine kinase, LiaR is a response regulator, and LiaF is a transmembrane protein of unknown function. Our previous data indicated that deletion of isoleucine in position 177 of LiaF in a laboratory strain (OG1RF) resulted in DAP tolerance and changes in membrane architecture. Here, we aim to study the role of LiaF in DAP resistance.
Methods: We generated *liaF* mutants in *E. faecalis* OG1RF and OG117 (derivate of OG1RF) harboring: *i)* four stop codons leading to premature truncation of the protein (OG117*liaF*$_{11\text{-}14}$*), and *ii)* an allele coding for deletion of isoleucine in position 177 (OG1RF*liaFΔ$_{177}$*). In addition, we complemented in *cis* the OG117*liaF*11-14* mutant using the CRISPR-Cas9 system with wild type LiaF. We also complemented OG1RF*liaFΔ$_{177}$ in trans,* using the nisin induced vector pMSP3535. DAP MICs were performed using E-test. In addition, broth microdilution DAP MIC were made in presence/absence of the N-terminal. We evaluated cell membrane anionic phospholipid (AP) microdomain localization using 10-*N*-nonyl-acridine-orange (NAO) and determined activation of LiaFSR by evaluating surface exposure of LiaX by ELISA. Lastly, we used the bacterial adenylate cyclase two-hybrid system (BACTH) to study the protein-protein interaction between LiaF-LiaS and LiaFΔ$_{177}$-LiaS.
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Aim

To study the role of LiaF in DAP resistance in *Efs*.

Background

DAP resistance (DAP-R) in *E. faecalis* has been associated with mutations in the LiaFSR system that play a critical role on orchestrating the cell envelope adaptive response to antibiotics (1). The LiaFSR system consists of LiaF, a transmembrane protein LiaS, a histidine kinase and LiaR, the response regulator (1-3). Previously, we found that the emergence of DAP-R in a patient after DAP therapy was associated with a *liaF* mutation, resulting in the single deletion of an isoleucine at aminoacid 177 (1). This finding suggests that LiaF and the C–terminal region of the protein are crucial in controlling activation of the LiaFSR system (1,4). Protein structure prediction of LiaF indicates four putative transmembrane domains with a cytoplasmic C-terminal tail with a stretch of 152 aa that contains four isoleucines at positions 176 to 179. Therefore; we hypothesize that LiaF modulates the LiaFSR system and the region harboring the isoleucines represent a key regulatory region.

Methods

Strains	DAP MIC (µg/ml) E-test	Features
OG1RF	2	DAP susceptible laboratory strain of Efs
OG1RF *liaF$_{\Delta 177}$*	2	Deletion of an isoleucine at position 177
OG1RF *liaF$_{\Delta 177}$* (pMSP3535)	2	Deletion of an isoleucine at position 177 with empty vector pMSP3535
OG1RF *liaF$_{\Delta 177}$* (pMSP3535::*liaF*)	2	Deletion of an isoleucine at position 177 with complementation *in trans* using pMSP3535::*liaF*
OG117	1.5	DAP susceptible laboratory strain of Efs derivative of OG1RF used for cris-cas9 system
OG1RF *liaF*$_{11\text{-}14}$*	1.5	Insertion mutant of four stop codons in LiaF at position 11-14 using crisp-cas9 system
OG1RF *liaF$_{*11\text{-}14}$:: liaF*	1.5	Complemented strain with LiaF using crisp-cas9 system

- ✓ ~~Mutant construction using crisp-cas 9 system and PheS system~~
- ✓ DAP E-test / DAP Microdilution MICs with LiaX N-terminal protein
- ✓ Localization of anionic phospholipids microdomains using 10-nonyl-acridine-orange (NAO) staining.
- ✓ Activation of LiaFSR by evaluation of surface exposure of LiaX by ELISA using LiaX N- terminal antibodies.
- ✓ Evaluation of LiaS-LiaF, LiaS-LiaFΔ177 and LiaF –LiaR protein-protein interaction using the bacterial adenylate cyclase two-hybrid system (BACTH).
- ✓ β-Galactosidase assay

Results

Localization of anionic phospholipid microdomains by NAO

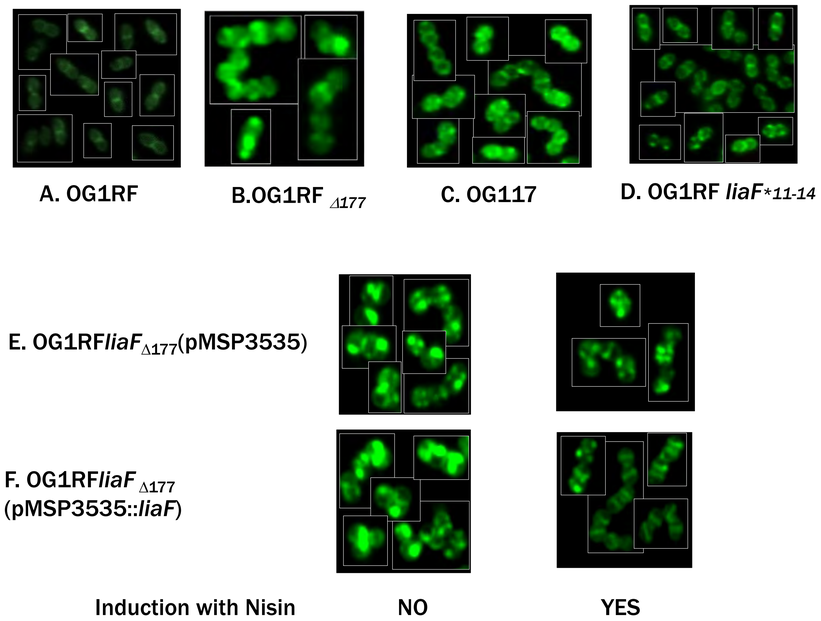

Figure 1. Staining of representative cells of *E. faecalis* OG1RF – OG117 and derivatives with NAO. Panels A, B, C and D the anionic phospholipid microdomains in septum of the cells in OG1RF, OG117 and it's derivatives. Panel E OG1RF *liaFΔ177 (pMSP3535)* mutant exhibits phospholipid microdomains located away from the main septum and distributed throughout the cells. Panel E, mutant OG1RF *liaFΔ177* complemented *in trans with* LiaF using pMSP3535 under the induction of Nisin 50 ng/ml after 6 hours.

Positive protein interaction between of LiaS-LiaF by BACTH

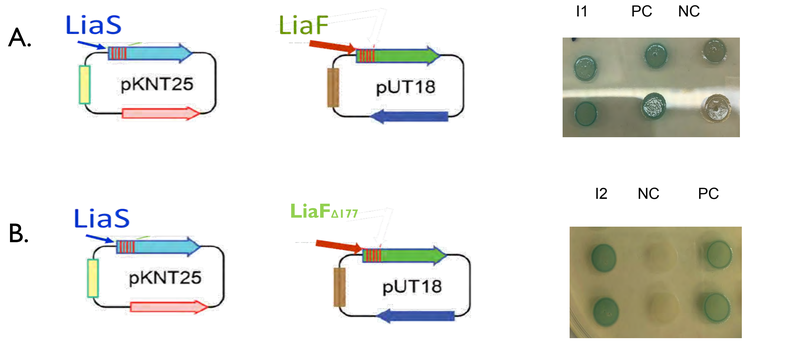

Figure 2. Bacterial Two Hybrid system of LiaF and LiaS protein-protein interactions. A-B. Interaction of LiaS-T25-LiaF-T18 and LiaS-T25-LiaFΔ177-T18. LiaS and LiaF, LiaF$_{177\Delta}$ were fused at N-terminus of T25 and pUT18. Blue colored colonies in LB/X-GAL-IPTG plates indicates a positive interaction between LiaS-LiaF (I1) and LiaS-LiaF$_{\Delta 177}$ (I2). Negative control (NC) interactions with empty vectors (pKNT25+pUT18).Positive control (PC) interaction with pKT25-zip + pUT18C-zip.

Quantification of LiaS-LiaF and LiaS-LiaFΔ177 by β-galactosidase assay

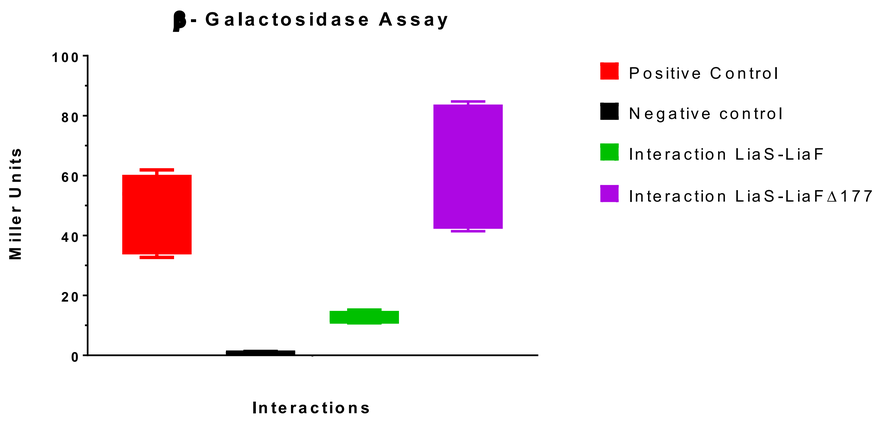

Figure 3. Quantification of the interaction between LiaS-LiaF and LiaS-LiaFΔ177 was measure by β- galactosidase assay. The positive control was the leucine zipper fused to T25 and T18 motif respectively. Negative control empty vectors (pKNT25+pUT18). The measurements of the interactions were carried out by three biological replicates.

Results

Is LiaF possible receptor for LiaX?

DAP Microdilutions with and with LiaXN–terminal protein

Strain	DAP MIC	DAP MIC +LiaX N-terminal protein (50nM)
OG117	2	8
OG1RF *liaF*$_{11\text{-}14}$*	2	2
OG1RF *liaF*1$_{11\text{-}14}$::liaF*	2	4

LiaX is highly detected in the mutant OG1RF$_{\Delta 177}$

ELISA surface expression of LiaX using N-terminal Antibodies

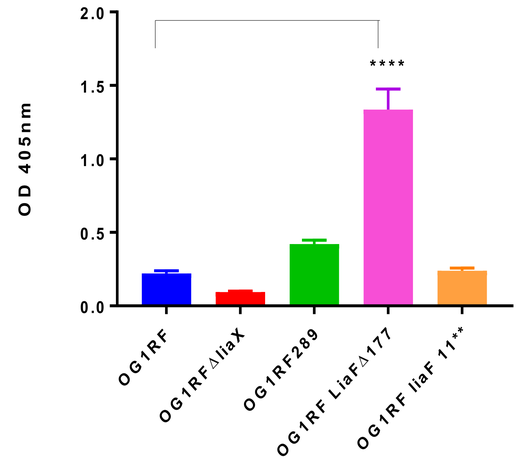

Figure 4. Detection of LiaX on cell the surface from *liaF* mutants. ELISA of LiaX was performed using antibodies against LiaX N-terminus. The strain OG1RF LiaF$_{\Delta 177}$produces higher levels of LiaX compared to other strains. The mean values are from 3 independent experiments with 12 replicates each. ****P < 0.0001 (2-tailed t test).

Conclusions

- Major changes in cell membrane remodeling with redistribution of anionic phospholipid micro-domains away from the septum was observed in the strain OG1RF LiaFΔ177.
- High levels of LiaX secretion were noted in OG1RF LiaF$_{\Delta 177}$ compared to the WT OG1RF, suggesting an important regulatory role of the stretch of this 4 isoleucines.
- A protein-protein interaction between LiaF and LiaS was detected.
- A stronger interactions was detected between LiaS- LiaF$_{\Delta 177}$ compared with LiaS-LiaF.
- LiaF seems to play a role as an activator of the LiaRSR system through a C-terminal region that involves a stretch of 4 isoleucines.

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Funding Source

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Genomic Characterization and Epidemiology of the Cefazolin Inoculum Effect in Methicillin-susceptible *S. aureus* from severe infections in patients in hospitals in Colombia.

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Background: Cefazolin is an alternative to antistaphylococcal-penicillins in the treatment of MSSA severe infections. However, the cefazolin inoculum effect (CzIE) could limit the therapeutic efficacy of cefazolin in deep-seated MSSA infections. The CzIE is associated to therapeutic failure and increased mortality. BlaZ-2 allotype (from BlaZ-type A) and clonal lineage ST30 were predominant molecular characteristics in MSSA exhibiting the CzIE from Latin-American hospitals. Recently, we have developed a rapid nitrocefin–based test and a Machine Learning (ML) model to identify MSSA showing the CzIE. On the other hand, accessory gene regulator (*agr*) genotypes have been potentially associated with the CzIE. The aim of this study was to characterize the CzIE in MSSA from severe infections recovered in hospitals in Colombia.

Methods: The MSSA isolates were recovered from 26 cases of severe infections (73% from bloodstream) in patients from four hospitals located in Bogota, between 2019-2021. Cefazolin MICs were determined at standard and high bacterial-inoculum by broth microdilution and rapid CzIE test was evaluated in the isolates. Whole-genome sequencing (WGS) was performed for identification of BlaZ-types, BlaZ-allotypes, *agr* genotypes, and Sequence types (ST). The *bla* cassette was used to predict the CzIE with previously developed (ML) model based on a Random Forest and kmers of size 17. Additionally, we obtained a core genome phylogenetic tree of the assemblies with RAxML.

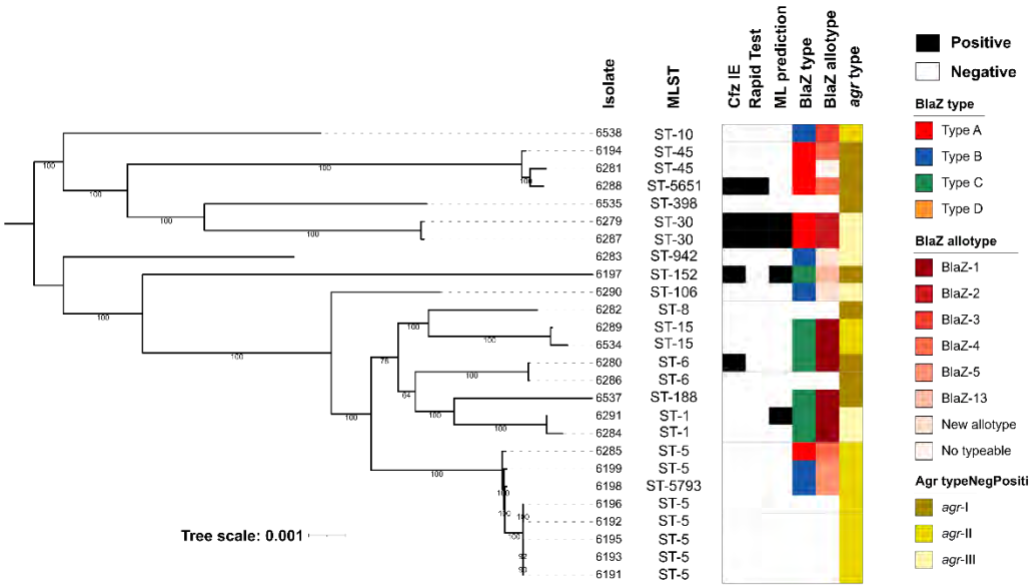
Results: The CzIE was identified in 19% (n=5) of 26 MSSA. Among the MSSA exhibiting CzIE, type A BlaZ was the most prevalent (60%), and BlaZ-2 was the most frequent allotype identified in 40% of isolates. The rapid test identified 3 true-positive isolates and 2 false-negatives (from BlaZ types A and C, respectively) among 5 CzIE-positive isolates. Whereas correctly identified all 21 MSSA lacking the CzIE. The phylogenetic tree showed a wide diversity of strains (15 different ST within 6 clonal complexes (CC), (Fig 1), with ST30 as the frequent lineage (33%) among the CzIE-positive MSSA. Further, *agr*-I was identified in 60% of the CzIE-positive MSSA. Moreover, the CzIE predicted by the ML model over the *blaZ* positive strains, showed a specificity of 92%, and sensitivity of 60%, with a precision and accuracy of 75% and 83%, respectively.

Conclusions: The CzIE is an important phenotype in MSSA from severe infections in Colombian hospitals, displaying similar molecular characteristics as previously documented in MSSA from Latin-American hospitals. Of note, *agr*-I genotype was detected in MSSA exhibiting CzIE. Further,

our results show the potential of ML prediction models to detect, based on sequence data, phenotypes not commonly tested in the clinical laboratory.

Acknowledgements: Minciencias 130880764150 and Grant 887 Young Researchers and Innovators in Medicine, and project PSI-2020-021 Universidad El Bosque

Figure 1. Core genome maximum likelihood phylogenomic tree of 26 MSSA genomes. Genomic characterization of the genomes is shown: MLST, CzIE phenotype, phenotypic rapid test and Machine Learning (ML) based prediction positive results are shown in black boxes. Characterization of *agr* types and BlaZ types and allotypes are shown in the last columns



Genomic Characterization and Epidemiology of the Cefazolin Inoculum Effect in Methicillin-susceptible *S. aureus* from severe infections in patients in hospitals in Colombia

Sandra Rincón[1], Lina P. Carvajal[1], Rafael Ríos[1], Natalia Muñoz[1], Lorena Diaz[1], Iván F. Gutiérrez[2], Cesar A. Arias,[3,4], and Jinnethe Reyes[1]

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Background

Cefazolin is an alternative to antistaphylococcal-penicillins in the treatment of Methicillin Susceptible *Staphylococcus aureus* (MSSA) severe infections (1). However, the cefazolin inoculum effect (CzIE) could limit the therapeutic efficacy of cefazolin in deep-seated MSSA infections (2).

The CzIE is associated to therapeutic failure and increased mortality in patients with MSSA bacteremia (3-5)

BlaZ-2 allotype (from BlaZ-type A) and clonal lineage ST30 were predominant molecular characteristics in MSSA exhibiting the CzIE from Latin-American hospitals (6).

Recently, we have developed a rapid nitrocefin–based test (7) and a Machine Learning (ML) model (8) to identify MSSA showing the CzIE. On the other hand, accessory gene regulator (agr) genotypes have been potentially associated with the CzIE (9).

Aim

The aim of this study was to characterize the CzIE in MSSA from severe infections recovered in hospitals in Colombia

Methods

Figure 1. Country and city were four hospital are located

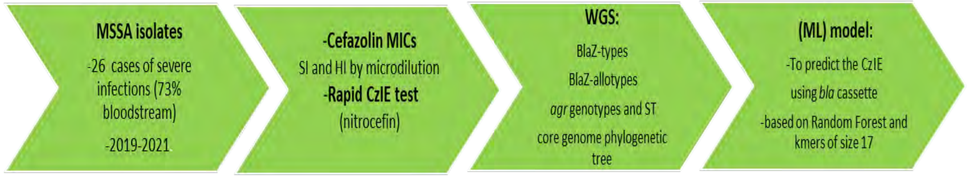

Figure 2. Methods used in this study

Results

- The CzIE was identified in 19% (n=5) of 26 MSSA

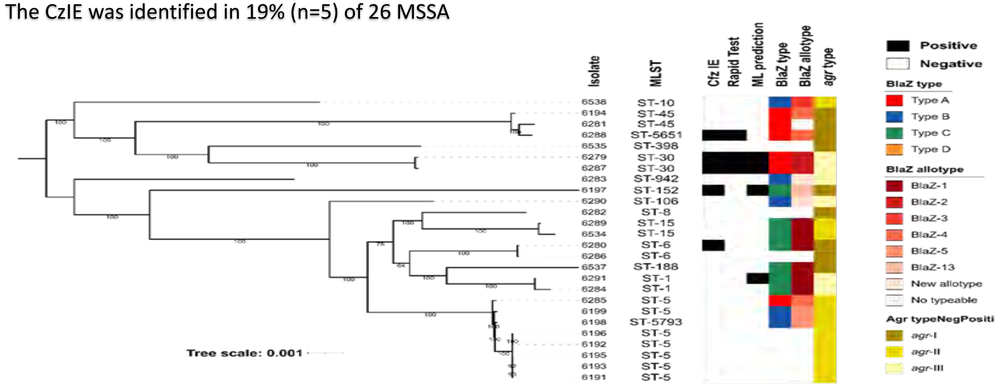

Figure 3. Core genome maximum likelihood phylogenomic tree of 26 MSSA genomes.

- The CzIE predicted by the ML model showed: specificity of 92% and sensitivity of 60% with a precision of 75% and 83% of accuracy

Conclusions

- The CzIE is an important phenotype in MSSA from severe infections in Colombian hospitals, displaying similar molecular characteristics as previously documented in MSSA from Latin-American hospitals.
- Of note, although *agr*-II was the most commonly identified among the isolates, the *agr-I* genotype was detected in MSSA exhibiting the CzIE.
- Further, our results show the potential of ML prediction models to detect, based on sequence data, phenotypes not commonly tested in the clinical laboratory.

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Acknowledgements

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Candida Sternal Wound Infections After Cardiac Operations: Uncommon but Deadly

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Background:
Sternal wound infections (SWI) can be a devastating complication of cardiac surgery resulting in prolonged hospital stays, multiple operative procedures, and poor outcomes. The majority of SWI are bacterial infections including *Staphylococcus aureus* and gram negative bacilli. *Candida* species are a less common cause of SWI, and *Candida* SWI is not well described in current literature. Advances in surgical technique and availability of new antifungal agents warrant a re-assessment of Candida SWI.

Goals:
The purposes of this study were (1) to describe clinical characteristics, management and outcomes of *Candida* SWI and (2) to compare the risk factors and outcomes for *Candida* SWI to bacterial SWI after cardiac surgery.

Methods:
Our study retrospectively reviewed medical records of 41 patients who had *Candida* SWI after cardiac surgeries between 2013 and 2020 at our medical center. After review of *Candida* SWI charts, we queried our adult cardiac surgery database to obtain data from 76 patients with bacterial SWI for comparison. We defined superficial SWI as positive culture isolates involving the skin or subcutaneous tissues, deep SWI as involving deep soft tissues or bone, and mediastinitis as involving the mediastinum.

Results:
Of 41 *Candida* SWI patients, the average age was 60.1 years old, 56.1% were male, and 70.7% were Caucasian. Some relevant comorbidities included prior history of cardiac surgery (46.3%), heart failure (65.9%), and diabetes (58.5%). Superficial SWI was seen in 9.8%, deep SWI was seen in 70.7% and mediastinitis was found in 19.5% of cases. *Candida* SWI was diagnosed after an average of 123.6 days after cardiac surgery. *Candida albicans* was most common at 70.7% of cases. Bacterial co-infections were found in 53.7%. Longer bypass and operative times for the initial cardiac surgery were found to be positively correlated to disease severity, which led to longer hospitalizations. Clinical cure rate was 100% in superficial SWI, 72.4% in deep SWI and 25.0% in mediastinitis. Overall mortality was 29.3%: 25% in superficial SWI, 24.1% in deep SWI and 50.0% in mediastinitis. When compared to bacterial SWI in the database, significant risk factors found to associated with *Candida* infection included: prior history of cardiac surgery (46.3% vs. 7.9%, odds ratio (OR): 5.9; 95% confidence interval (CI): 2.2-15.9), heart failure at time of cardiac surgery (65.9% vs. 11.8%, OR: 5.6; 95% CI: 2.4-12.9), and prolonged postoperative antibiotic use for > 48 hours (39.0% vs. 3.0%, OR: 9.9; 95% CI: 2.7-35.9). Mortality rates are also found to be higher with *Candida* infections when compared to bacterial infections (29.3% vs. 1.3%, OR: 22.2; 95% CI 2.8-177.2).

Conclusion:
This study found that *Candida* SWI often presents as a serious complication of extensive cardiac surgery with prolonged postoperative ICU stay. Prior history of cardiac surgery and heart failure, prolonged surgeries, and complicated postoperative course were significant risk factors for the development of *Candida* SWI when compared to bacterial SWI. *Candida* SWI were associated with higher mortality rates.

Candida Sternal Wound Infections After Cardiac Operations: Uncommon but Deadly

Seo JM[1], Louie BJ[1], Phe K[2], Ghanta RK[3], Trautner BW[4,5], Fukuta Y[4]

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Introduction

- Sternal wound infections (SWI) can be a devastating complication of cardiac surgery resulting in prolonged hospital stays, multiple operative procedures, and poor outcomes.
- The majority of SWI are bacterial infections, including *Staphylococcus aureus* and gram negative bacilli.
- *Candida* species are a less common cause of SWI, and *Candida* SWI is not well described in current literature.
- Advances in surgical technique and availability of new antifungal agents warrant a re-assessment of Candida SWI.

Purpose

- Describe the clinical characteristics, management, and outcomes of *Candida* SWI.
- Compare the risk factors and outcomes for *Candida* SWI to bacterial SWI after cardiac surgery.

Methods

- Retrospectively reviewed medical records of 41 patients who had *Candida* SWI after cardiac surgeries between 2013 and 2020 at our medical center.
- Queried our adult cardiac surgery database to obtain data from 76 patients with bacterial SWI for comparison.
- Defined superficial SWI as positive culture isolates involving the skin or subcutaneous tissues, deep SWI as involving deep soft tissues or bone, and mediastinitis as involving the mediastinum.

Results

Table 1: Clinical Characteristics & Comorbidities of *Candida* SWI Cases (n=41)

Average age (years)	60.1
Males	56.1%
Caucasian	70.7%
Prior history of cardiac surgery	46.3%
Heart failure	**65.9%**
Diabetes mellitus	58.5%
Superficial SWI	9.8%
Deep SWI	**70.7%**
Mediastinitis	19.5%
Average time until diagnosis of SWI (days)	**123.6**
Bacterial co-infection	53.7%
Average LOS after infection onset (days)	**47**

Table 2: Comparison between Candida and Bacterial SWI Cases

	Candida SWI (n=41)	Bacterial SWI (n=76)	Odds Ratios (OR)	Confidence Interval (CI)	P-value
Prior history of cardiac surgery	46.3%	7.9%	5.9	2.2 - 15.9	0.0005
Heart failure	65.9%	11.8%	5.6	2.4 - 12.9	0.0001
>48h post-op antibiotic use	39.0%	3.0%	9.9	2.7 - 35.9	0.0005
Overall mortality rate	29.3%	1.3%	22.2	2.8 - 177.2	0.0034

Table 3: *Candida* species associated with SWI (including some cases with co-infection)

Candida albicans	*Candida glabrata*	*Candida parapsilosis*	*Candida tropicalis*	*Candida lusitaniae*
70.7%	12.2%	12.2%	9.8%	0.2%

Results (cont.)

Table 4: Outcomes between different types of *Candida* SWI Cases (n=41)

	Superficial SWI (n=4)	Deep SWI (n=29)	Mediastinitis (n=8)
Clinical cure rate	100%	72.4%	**25.0%**
Overall mortality rate	25%	24.1%	**50.0%**

Discussion

- A medical history of heart failure, prior cardiac surgery, and prolonged post-operative antibiotic durations were statistically significant risk factors for *Candida* SWI over bacterial SWI.
- Deep SWI were the most common type of *Candida* SWI.
- Progressively deeper *Candida* SWI had lower clinical cure rates and higher overall mortality rates.

Conclusion

- *Candida* SWI often presents as a serious complication of extensive cardiac surgery with prolonged postoperative ICU stay.
- Prior history of cardiac surgery and heart failure, prolonged surgeries, and complicated postoperative course were significant risk factors for the development of *Candida* SWI when compared to bacterial SWI.
- *Candida* SWI were associated with higher mortality rates.

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Trick and Treat: Intermittent Antibiotic Dosing to Eradicate Persister Bacteria

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Background: A small fraction of bacteria use persistence as a strategy to survive exposure to antibiotics. Persistence is conferred by dormancy (non-growth): while bacteria remain dormant they cannot be killed by conventional antibiotics, unless the bacteria leave dormancy (recommence growth) and become susceptible to antibiotics again. Persisters are implicated in many chronic infections such as recurrent urinary tract infection or cystic fibrosis, and are difficult to eradicate with existing antibiotics. Moreover, prolonged persistence favors the emergence of complete antimicrobial resistance. The state-of-the-art is prolonged treatment of persisters with antibiotics, hoping for eventual eradication with the help of a patient's immune system. This strategy is not as successful as one would hope for.

Hypothesis/Goals: Detailed computer simulations with calibrated models have lead us to the hypothesis that persisters can be eradicated by an alternating antibiotic dosing regimen, as follows: Expose bacteria to a repeated cycle of (a) high enough antibiotic concentration that kills all susceptible cells, before they become dormant, and (b) a low antibiotic concentration at an appropriate time within the cycle, enticing persisters to recommence growth and be susceptible to the antibiotic.

Methods: Computational – MATLAB ® is used for modelling and optimisation. Experimental – For *in vitro* experiments, an *E. Coli*. (transformed with GFP) culture is treated with Ampicillin. All cultures are incubated at 37°C. Cycle: (a) Expose bacteria to Ampicillin (100 μg/ml) for 3h, (b) wash treated cells and grow in fresh media for 2h. Bacteria population size is assessed by colony counting on LB-Agar plates. A control population exposed at constant Ampicillin concentration is also used.

Results: The hypothesis is confirmed and repeatable. The alternating Ampicillin concentration eradicates all bacteria within 13 h, whereas the constant concentration in the control fails to do so.

Conclusions: Alternating dosing of antibiotics offers a promising potential for treatment of persistent bacterial infections. We are currently exploring (a) optimal switching times between high and low antibiotic concentrations, and (b) randomised switching times

Acknowledgements: Help from graduate students in Conrad and Orman labs is greatly appreciated. This research was partly supported by UH GEAR grant 2091-H0067-B0421-I113494 (ST 61553).

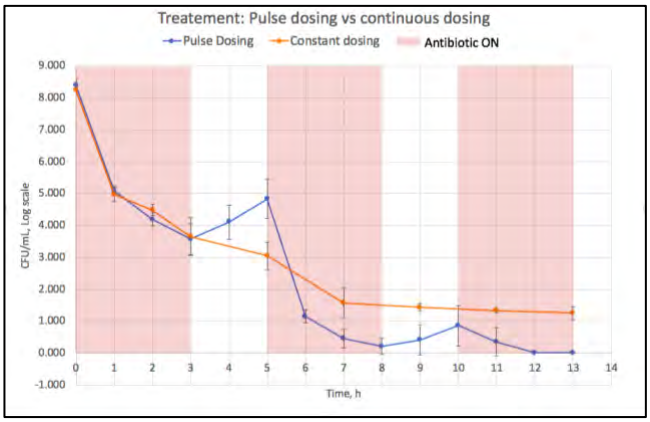

Figure 1 Pulse (alternating) dosing vs constant dosing. 3 h Ampicillin treatment followed by 2 h of growth in fresh media.

Trick and Treat: Intermittent Antibiotic Dosing to Eradicate Persistent Bacteria

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Motivation

Modified from (Epstein et. al, 2007)

Bacterial persistence

- Hibernating enemy
- Strategy to survive antibiotics
- Slow or no growth state
- Different from resistance

Persistent bacteria lead to

- Relapse of infections (Recurrent urinary tract infections, Tuberculosis)
- Multidrug tolerance
- Catalyst for resistance

Therapeutic ideas to eradicate persisters

Use existing antibiotics

- Manipulate dosing regimens
- Based on population dynamics
- Challenges: Optimal dosing frequency, Personalization

Develop New drugs

- Inhibit persister formation, Re-sensitize persisters to normal cells
- Mechanism based
- Challenges: identifying multiple-mechanism and non-toxic drugs

Objective

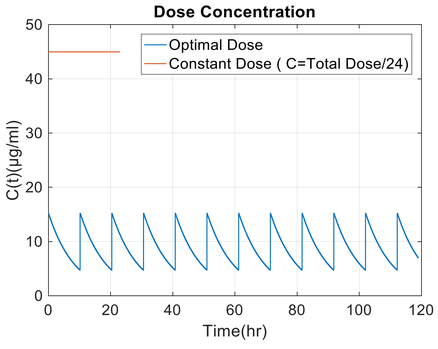
Test its *in vitro* efficacy of idea for eradicating persistent bacteria via intermittent dosing.

Simulations

$$\frac{dn}{dt} = K_n n + bp$$

$$\frac{dp}{dt} = an + K_p p$$

n = normal cells , p = persister cells
K_n = net kill/ growth rate of normal cells
K_p = net kill/ growth rate of persister cells
a = switch rate from n to p state
b = switch rate from p to n state



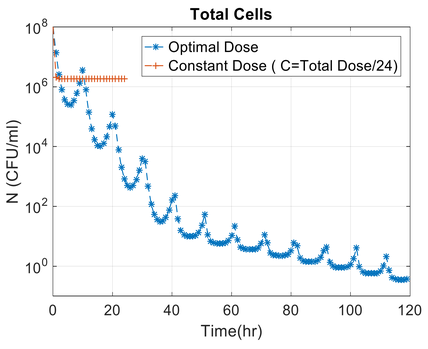


Figure 2. Simulations for E. Coli treatment with Ampicillin constant dosing using (Balaban et. al., 2004) model

In vitro tests and model calibration

Materials

Bacteria Strain: *Escherichia coli* (wild type);

Antibiotic: Ampicillin;

Culture media: Lysogeny Broth (LB), LB agar plates

Methods

- Overnight (24 h) cultures prepared from frozen glycerol stock (-80 ºC)
- Primary culture (from stationary overnight culture) with 100-fold dilution
- Cells washed with PBS buffer solution to remove the antibiotics
- Cells serially diluted in PBS using 96-well plates, spotted on LB agar, incubated at 37 °C for 16 h to enumerate CFUs
- MATLAB Ode solvers and optimization toolboxes.

Results

Constant dosing

- Colonies seen on all replicates at the end of treatment (5-100)
- Kill rates decline rapidly and flattens towards the end

Pulse dosing

- No colony observed after last kill cycle for all replicates
- Even with 1h of growth in fresh media kill rates increase manifold

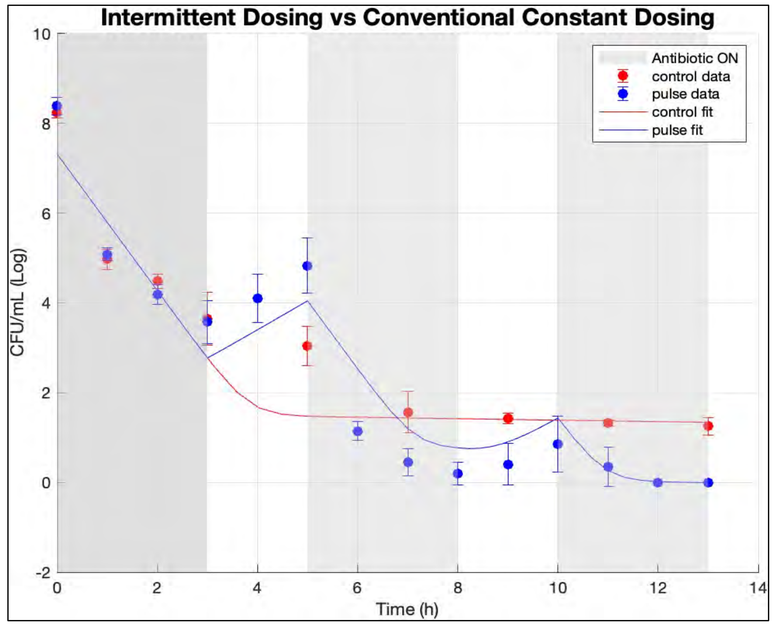


Figure 2. E. Coli. treated with Ampicillin with constant dosing and intermittent dosing

Conclusion

- Intermittent dosing outperforms constant dosing and achieves eradication of persistent bacteria

Future Work

- Test for other bacteria strains and classes of antibiotics
- Design robust/optimal regimen strategy: antibiotic concentration, frequency of administration
- Improve predictive accuracy of dynamic model

Acknowledgement and References

I greatly appreciate graduate students in Conrad Lab and Orman lab for helping me with bacterial experiment and microscopy training. UH GEAR grant 2091-H0067-B0421-I113494 (ST 61553)

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The TAILΦR Initiative at Year One: Personalized Medicine for Dynamic Infections

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Background/Goals The multidrug-resistance (MDR) crisis creates patients stuck in cycles of infection, treatment, and relapse. TAILΦR seeks to break that cycle by pioneering phage therapy as personalized medicine. Through the Food and Drug Administration's (FDA) investigational new drug (IND) and expanded access mechanisms, TAILΦR creates bespoke phage cocktails targeting MDR ESKAPE pathogens. However, there are many challenges between phage discovery and treatment. Here, we describe the technical and scientific challenges for generating effective personalized phage cocktails while simultaneously navigating the U.S. regulatory framework to hit a microbial "moving target". Lessons learned inform best practice guidelines.

Methods Clinical isolates were sent to TAILΦR Labs, with virulent phages either identified by screening our anti-ESKAPE phage bank or isolated *de novo* from environmental sources then purified using cesium chloride gradients. Each purified phage preparation was sequenced and analyzed for purity and safety. Selected phages were mixed into a cocktail and endotoxin removed via Endotrap columns. During the two-week USP 71 sterility testing, cocktails were characterized by endotoxin content, titer, killing assays, storage longevity, and antibiotic synergy. All data was handed over to the physician for IND/expanded access filing with consultation from TAILΦR. Once granted permission to proceed, investigational pharmacies received and formulated the cocktail into individual doses for IV administration. Additional clinical science, including the testing of phage neutralization by the innate and adaptive immune system were assessed when possible.

Results TAILΦR responded to 47 requests for a phage hunt, for which we received 40 clinical isolates and generated 150 novel phages against 11 bacterial species. Most common indications requested were urinary tract infection/prostatitis, left-ventricular assist device infections, and bacteremia. From those 40 cases, 12 patients received treatment. The patient's initial infection cleared in all cases with one mild adverse event reported.

Conclusions The months between physician request and cocktail administration are substantial when considering that the patient's bacterial population is capable of transduction, transformation, conjugation, and *de novo* mutation – actions that undermine current antibiotic treatments. Thus, the true potential of phage therapy will not be realized until discovery, manufacturing, and regulatory efforts harmonize to rapidly deliver personalized cocktails in a manner that curtails real-time evolution. TAILΦR is addressing barriers by developing good manufacturing practices (GMP) to significantly reduce time-to-treatment and curating cocktails that anticipate bacterial resistance. Additionally, TAILΦR will reduce future attrition by screening for patient need, physician experience, capabilities of the institution and associated pharmacy, and phage activity in the host microenvironment.

Acknowledgements This work was funded by U19 AI157981, Robert and Helen Kleberg Foundation, Mike Hogg Foundation, and BCM seed funds awarded to A.M.

Brochure Website

Tailored Antibacterials and Innovative Laboratories for phage (Φ) Research

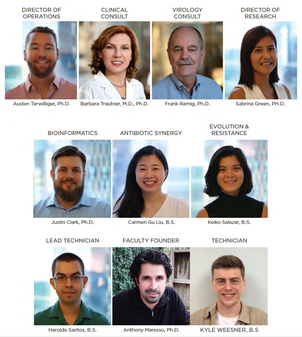
Baylor College of Medicine

The TAILΦR Initiative at Year One: Personalized Medicine for Dynamic Infections

Terwilliger A[1], Hernandez Santos H[1], Clark J[1], Green S[1], Weesner K[1], Ramig F[1], Trautner B[2], Maresso A[1]

1.TAILΦR Labs, Molecular Virology and Microbiology Department, Baylor College of Medicine
2.Michal E. DeBakey Veteran Affairs Medical Center

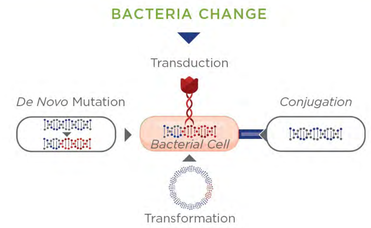
MEET YOUR TAILΦRS



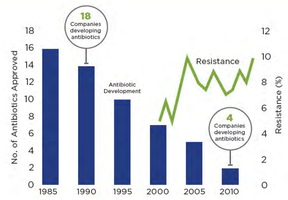
Background The multidrug-resistance (MDR) crisis creates patients stuck in cycles of infection, treatment, and relapse. TAILΦR seeks to break that cycle by pioneering phage therapy as personalized medicine. Through the Food and Drug Administration's (FDA) investigational new drug (IND) and expanded access mechanisms, TAILΦR creates bespoke phage cocktails targeting MDR ESKAPE pathogens. Here, we describe the technical and scientific hurdles for generating effective personalized medicines while simultaneously navigating the U.S. regulatory framework to hit a microbial "moving target". Lessons learned inform best practice guidelines.

Future Directions The months between physician request and cocktail administration are substantial when considering that the patient's bacterial population is capable of transduction, transformation, conjugation, and *de novo* mutation – actions that undermine current antibiotic treatments. Thus, the true potential of phage therapy will not be realized until discovery, manufacturing, and regulatory efforts harmonize to rapidly deliver personalized cocktails in a manner that curtails real-time evolution. TAILΦR is addressing barriers by developing good manufacturing practices (GMP) to significantly reduce time-to-treatment and curating cocktails that anticipate bacterial resistance. Additionally, TAILΦR will reduce future attrition by screening for patient need, physician experience, capabilities of the institution and associated pharmacy, and phage activity in the host microenvironment.

Why Bacteriophage (Phage) are Needed



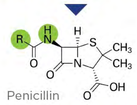
AND IT'S EXACERBATING A CRISIS! It takes around 10 years and $1 billion for a company to bring a new antibiotic to market. Bacteria quickly evolve resistance to these drugs, sometimes within months. This undercuts the economic incentives to invest in the discovery and development of new antibiotics. Unsurprisingly, as bacterial resistance has **increased**, the number of companies developing new antibiotics has **decreased**.



EITHER WE CHANGE THE WAY WE APPROVE AND REGULATE NEW DRUGS, OR WE DEVELOP AND APPROVE NEW DRUGS THAT CHANGE.

BACTERIA ARE REMARKABLY ADAPTABLE...AND THAT'S THE PROBLEM! Four intersecting mechanisms of change, what we call the mutagenic tetrasect, come together to facilitate the ability of bacteria to change and adapt. Each of these means of acquiring new DNA contributes to the success of bacteria in undermining our attempts to control them. Bacteria can mutate their way around antibiotics, vaccines, biocides, and engineering controls.

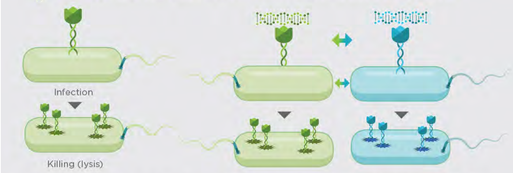
MEDICINES DON'T CHANGE



EVERY ANTIBIOTIC THAT TARGETS PATHOGENIC BACTERIA HAS A FIXED CHEMICAL STRUCTURE LIKE PENICILLIN ABOVE, upon which chemists can add a few more atoms (highlighted green circles) to make the antibiotic more effective. Eventually, bacteria mutate and find ways to overcome these new structures. In return, chemists develop new modifications of the same core structure. Bacteria become resistant once more, and so on. Eventually, chemists run out of places to make modifications!

THE SOLUTION IS BACTERIAL KILLERS THAT CHANGE!

PHAGE ARE THE MOST NUMEROUS REPLICATING ENTITY ON EARTH.



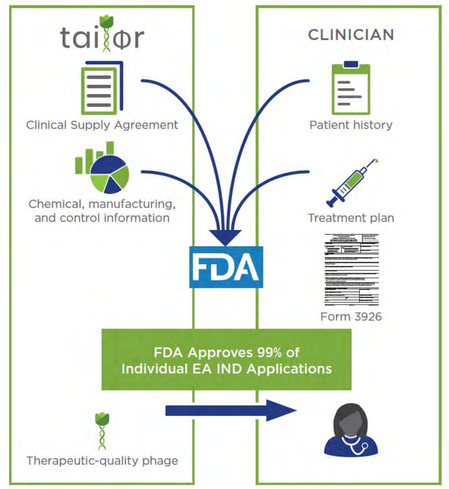
They are like a flu for bacteria, and specifically infect them. Phage replicate inside until they burst free, killing the cell.

Unlike chemical antibiotics, phage have DNA. By mutating their DNA, phage can evolve as quickly as bacteria.

TAILΦR can wield this evolution to develop the best bacterial killers quickly!

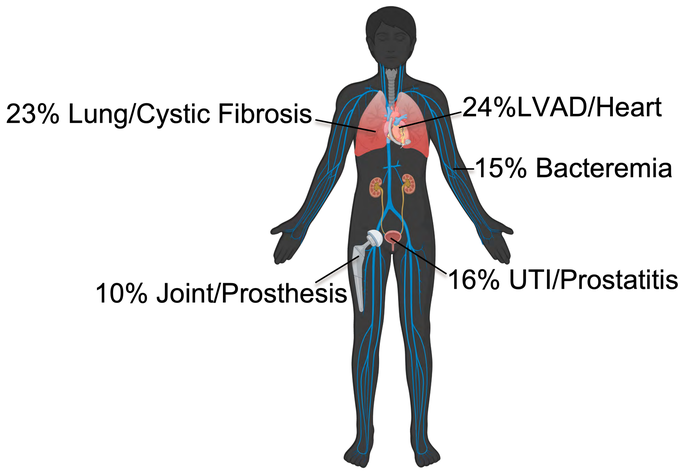
Investigational New Drug (IND) Application

EIND: For emergency use when a patient must be treated prior to a formal written submission to the FDA.

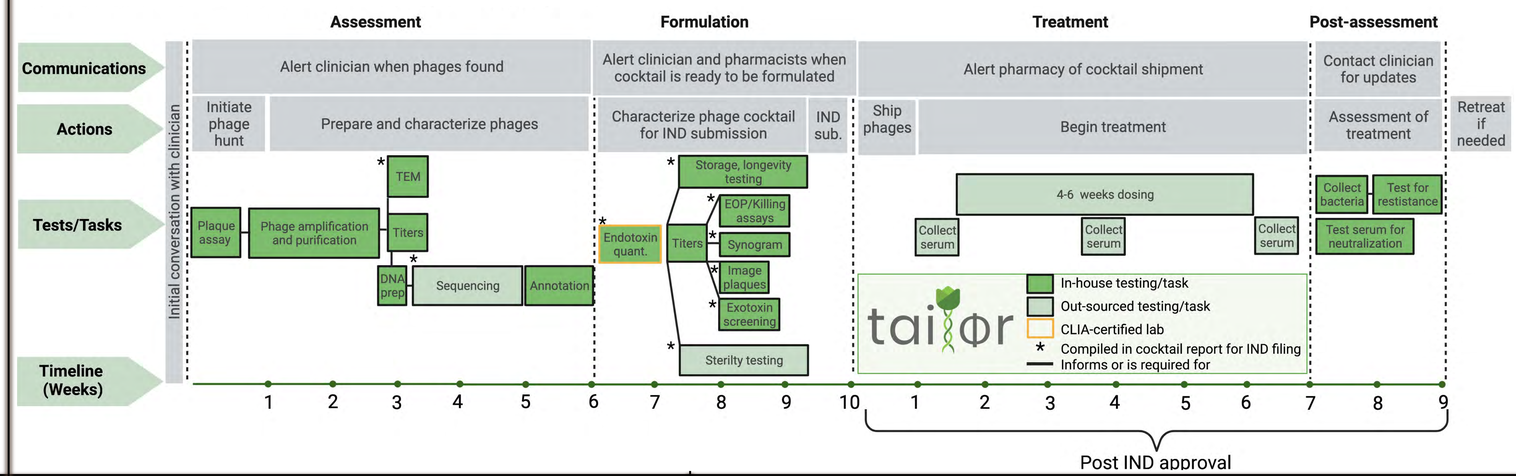


Important: An associated pharmacy capable of sterile manipulation is usually required

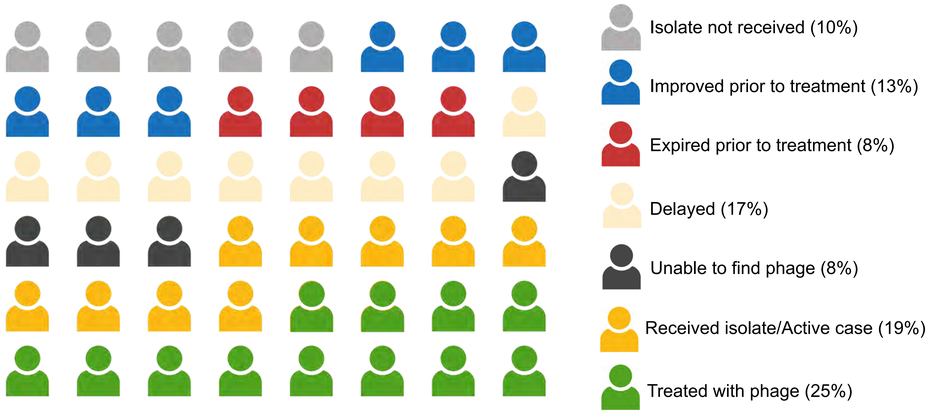
Indications Requested



Phage Therapy Pipeline



Case Attrition for 48 Requests

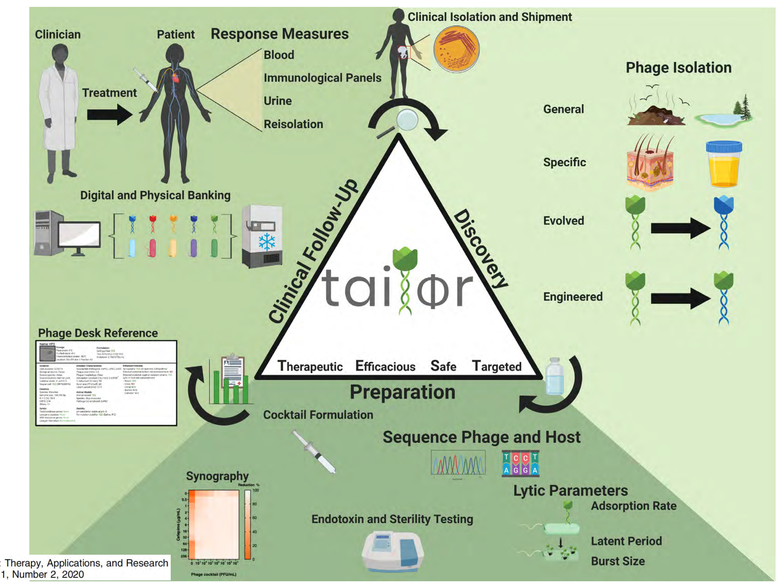


Current Phage Inventory

Host Species	# of phages	%
Achromobacter xylosoxidans	22	12.29%
Acinetobacter baumannii	4	2.23%
Burkholderia gladioli	1	0.56%
Enterobacter cloacae	9	5.03%
Enterococcus faecalis	1	0.56%
Enterococcus faecium	3	1.68%
Escherichia coli	37	20.67%
Klebsiella aerogenes	4	2.23%
Klebsiella pneumoniae	19	10.61%
Pseudomonas aeruginosa	32	17.88%
Staphylococcus aureus	3	1.68%
Staphylococcus pseudintermedius	17	9.50%
Stenotrophomonas maltophilia	23	12.85%
Enterococcus spp	4	2.23%
Grand Total	**179**	**100.00%**

Acknowledgments This work was funded by the Mike Hogg Foundation, The Robert and Helen Kleberg Foundation, Baylor College of Medicine seed funds, and U19 AI157981 from NIH/NIAID awarded to A.M.

A Personalized, TAILΦRED Future



PHAGE: Therapy, Applications, and Research
Volume 1, Number 2, 2020

The Integrative Conjugative Element ICESpyM92 Contributes to Pathogenicity of Emergent Antimicrobial-Resistant emm92 Group A Streptococcus

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Background: Mobile genetic elements (MGEs), such as plasmids, transposons, and *I*ntegrative *C*onjugative *E*lements (ICEs), are a major source of antimicrobial resistance (AMR) genes. AMR-encoding MGEs are widespread in the absence of antimicrobial pressure, raising the possibility that additional factors are involved in their maintenance and dissemination. For instance, AMR-encoding MGEs potentially contribute to bacterial virulence. MGEs can encode virulence genes alongside AMR elements, or virulence genes whose products alter antimicrobial susceptibility, or even gene content (e.g. transcriptional regulators) that can indirectly influence virulence and promote AMR maintenance. *Streptococcus pyogenes* (Group A Streptococcus, GAS) is an exclusively human pathogen that primarily colonizes the epithelia of the human throat and skin. Acquisition of MGEs in GAS contributes to enhanced virulence, clone emergence and niche specialization. GAS remain universally susceptible to β-lactams and thus have not, historically, been the focus of AMR research. However, recent epidemiological data reveal increasingly frequent GAS invasive disease in the United States attributable to macrolide-resistant strains. Whole genome sequencing analysis of invasive AMR strains has revealed that diverse MGEs encode AMR in GAS. Among these we described tetracycline and aminoglycoside resistance encoded in a highly conserved 65-kb ICE (ICESpyM92) among closely related and increasingly frequent *emm92* GAS strains nearly exclusively isolated from invasive or skin and soft tissue infections (SSTIs). The greater frequency of AMR relative to other circulating GAS isolates detected among contemporary *emm92* isolates and their strong association with severe disease suggests that *ICESpyM92* gene content, independent of AMR, contributes to disease phenotype.

Hypothesis: ICESpyM92 gene content contributes to GAS disease potential by influencing global gene expression.

Methods: GAS *emm92* isolates and isogenic mutants either *cis*-complemented with *ICESpyM92* (ICE+) or lacking the MGE (ΔICE), respectively, were compared using *in vitro*, *ex vivo* (human epithelial keratinocytes) and *in vivo* (mouse) models of subcutaneous infection to assess ICESpyM92-related streptococcal differential gene expression (DGE), invasion and virulence.

Results: Comparisons of representative contemporary *emm92* (ICE+) to historical (ΔICE) *emm92* and isogenic ΔICE and ICE+ mutants in these respective backgrounds indicate that the presence of ICESpyM92 enhances GAS virulence in a murine subcutaneous infection model. ICE+ strains generated higher infectious burdens during the acute phase of infection and more widespread host tissue damage. Measurement of *in vitro* and *ex vivo* DGE indicates ICESpyM92 influences GAS global gene expression in a background-dependent manner, including expression of multiple virulence factors (*emm*, *slo*) and genes linked to niche adaptation (*lctO*). Transcriptomic analysis further indicates that DGE solely related to core chromosomal differences in contemporary *emm92* relative to a historical (ΔICE) strain, independently of ICESpyM92, may also contribute to emergent *emm92* virulence.

Conclusions: Our data support the conclusion that ICESpyM92 contributes to GAS pathogenicity and provide evidence that a combination of ICESpyM92- and core genome-dependent DGE contributes to invasive disease phenotypes in emergent AMR *emm92* GAS. Our study links virulence and AMR on a unique MGE via MGE-related DGE, and highlights the importance of investigating associations between AMR-encoding MGEs and bacterial pathogenicity.

Acknowledgements: Research supported by NIH T32AI141349-01A1.

Contribution of an Integrative Conjugative Element (*ICESpyM92*) to virulence of emergent antimicrobial resistant Group A *Streptococcus*

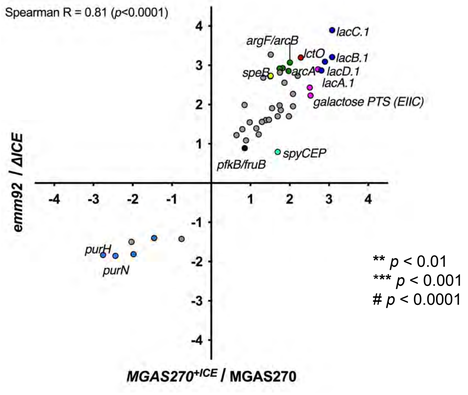
Vega LA[1], Sanson MA[1], Regmi S[1], Shah BJ[1], Cubria MB[1], Alamarat Z[1], Flores AR[1,2]

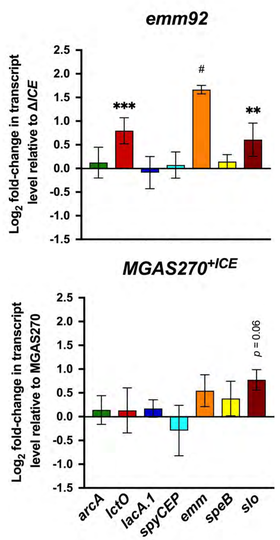
[1]Department of Pediatrics, [2]Center for Antimicrobial Resistance and Microbial Genomics (CARMiG), McGovern Medical School

Abstract

- *I*ntegrative *C*onjugative *E*lements (ICE) encode antimicrobial resistance (AMR) genes in many human bacterial pathogens.
- ICE can also encode virulence genes and/or alter global gene regulation, leading to enhanced pathogenicity.
- Potential contribution to maintenance and propagation of AMR.
- A 65-kb ICE (ICESpyM92) conferring aminoglycoside and tetracycline resistance was detected in >98% of recently emerged type *emm92* Group A *Streptococcus* (GAS) invasive infection isolates.
- *Hypothesis: ICESpyM92 contributes to disease potential of GAS by altering streptococcal global gene expression.*
- Comparison of isogenic ICESpyM92 mutants indicates:
  - Emergent *emm92* GAS exhibit greater skin pathogenicity *in vivo*.
  - ICESpyM92 alters GAS global gene expression in a background-dependent manner.
  - ICESpyM92-associated and ICE-independent differential virulence gene expression *in vitro* and *ex vivo* in emergent *emm92.*

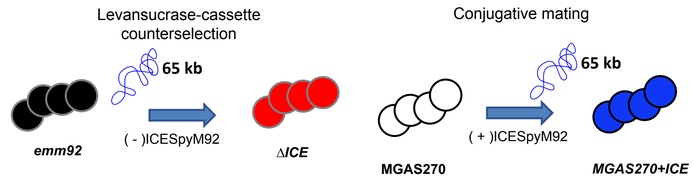
ICESpyM92-related expression of GAS virulence genes is background-dependent





- *In vitro* RNAseq (above) and qRT-PCR *ex vivo* (HEK-adherent GAS, right) analyses of ICESpyM92-dependent differential GAS gene expression.
  - ***lctO***: Lactate oxidase involved in host colonization
  - ***emm***: M-protein (immunomodulatory, antigenic)
  - ***slo***: Streptolysin O (cytotoxin)

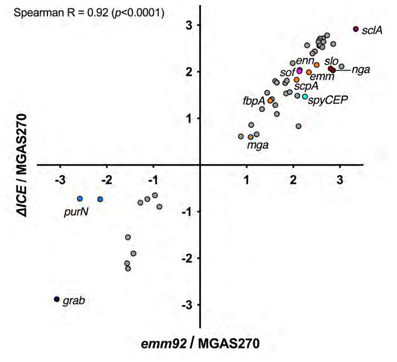
Isogenic ICE(+) and ICE(-) mutants generated in emergent, historical *emm92* for comparison

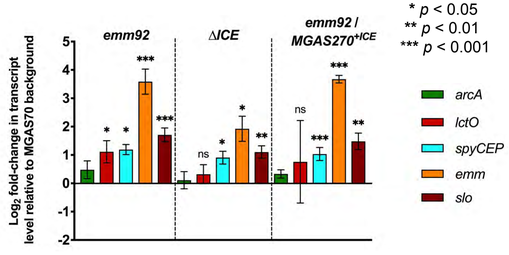


- Emergent *emm92* mutant lacking the MGE (Δ*ICE*) generated by sucrose counterselection of an integrated levansucrase cassette.
- Historical *emm92* (MGAS270) *cis*-complemented with *ICESpyM92* (*MGAS270*$^{+ICE}$) generated by conjugative mating of MGAS270.
- Isogenic mutants compared in skin and soft tissue infection models
  - *In vitro* human epidermal keratinocytes (HEKs).
  - *In vivo* subcutaneous mouse model.

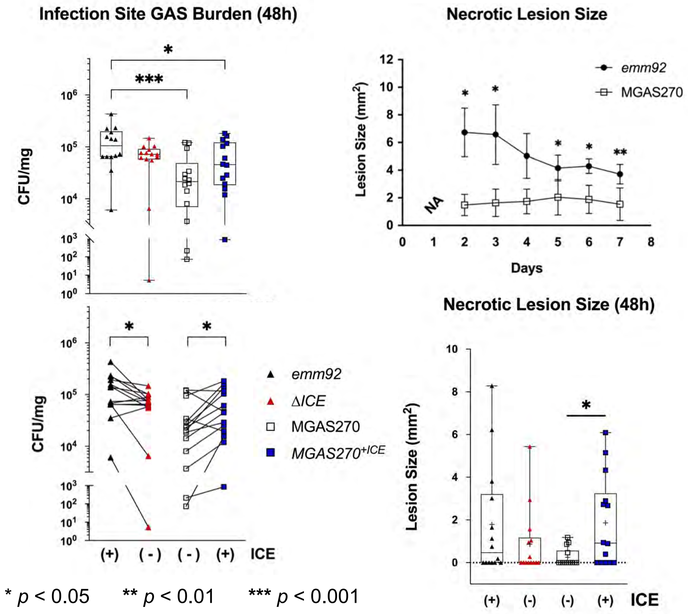
Emergent *emm92* exhibit ICESpyM92-independent differential expression of GAS virulence genes

- *In vitro* RNAseq (right) and *ex vivo* (HEK-adherent GAS, bottom) qRT-PCR analyses of differential GAS gene expression independent of the presence of ICESpyM92.
- GAS were allowed to adhere to cultured HEKs for 2 hours prior to cell washing, lysing, and RNA extraction.
- Differentially expressed genes in RNAseq analysis as exhibited >1.5 fold change in expression ($p < 0.05$, Bonferroni correction).
- An immunomodulatory protease (*spyCEP*) shows significantly enhanced expression in HEK-adherent emergent *emm92* GAS (qRT-PCR, ΔΔCT method, >1.5 fold change in expression, $p < 0.05$, student's t-test).





Emergent and historical *emm92* differ in skin infection site burden and tissue damage



- Pathogenesis of emergent (*emm92*), historical (MGAS10870) and isogenic ICESpyM92 mutant strains (Δ*ICE*, *MGAS270*$^{+ICE}$) were compared using a mouse model of subcutaneous infection.
- Bacterial burden assessed at 48 hours post-infection by enumerating CFU in tissue (left panels; Wilcoxon test for statistical significance).
- Dermal necrotic lesion size measured daily (right panels) and median lesion size compared to assess tissue damage (Mann-Whitney test for statistical significance).

Conclusions, Future Directions

Emergent *emm92* gene expression is distinct from antimicrobial-susceptible *emm92*, both in ICE-related and independent manners. Our work further defines the contribution of mobile genetic elements (MGEs) to bacterial pathogenicity by linking ICE-related differential gene expression and virulence with AMR on a unique MGE. Understanding the AMR-encoding MGE contribution to gene regulation and virulence of bacterial pathogens will advance efforts to combat emergence of AMR pathogens. Work was supported by Training Program in Antimicrobial Resistance (TPAMR, T32 AI141349 to L.A.V.) and R01 AI124216 (A.R.F.)

Phage Resistance Accompanies Reduced Fitness of Uropathogenic E. Coli in the Urinary Environment

Zulk JJ[1], Clark JR[1], Ottinger S[1], Ballard MB[1], Mejia ME[1], Mercado-Evans V[1], Heckmann ER[1], Sanchez BC[1], Trautner BW[2,3], Maresso AW[1], Patras KA[1,4]

1. Department of Molecular Virology and Microbiology, Baylor College of Medicine
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Corresponding author: Jacob Zulk, Department Molecular Virology and Microbiology, Baylor College of Medicine, 1 Baylor Plaza, Houston, Texas, E-mail: zulk@bcm.edu

Background: The estimated 7 million urinary tract infections (UTIs) occurring annually in the United States represent the most common cause of outpatient antibiotic prescriptions. UTIs are common in women, elderly individuals, and those with underlying medical conditions. The majority are caused by uropathogenic *E. coli* (UPEC) and are treated with antibiotics. However, alternative treatments are urgently needed due to growing antibiotic resistance and the negative impact of antibiotics on the healthy microbiota. Bacteriophages (phages), viruses that infect bacteria, are appealing alternatives due to their specificity for bacterial hosts and ubiquity in nature. As with antibiotics, bacteria can become resistant to phage. While several groups have investigated bacterial phage resistance mechanisms, no groups have evaluated the effects of resistance in the urinary tract environment.

Hypothesis/Goals: We hypothesized that UPEC would become resistant to phage ES17 through genomic changes and that phage resistance would negatively impact bacterial fitness in the urinary tract environment.

Methods: We isolated phage resistant UPEC by challenging UTI89, a well-characterized cystitis isolate, or DS566, a recent isolate from a patient with neurogenic bladder, with phage ES17 in liquid culture for 18 hours. We sequenced these bacteria to identify genetic mutations being made to evade phage killing and utilized *in vitro* and *in vivo* assays to evaluate the bacterial fitness costs associated with resistance to phage ES17.

Results: Through our investigation, we identified ES17 resistance arising in UTI89 and DS566 in both bacteriologic medium and in pooled human urine driven by mutations in LPS. These LPS-truncated bacteria display attenuated growth in urine, but that this phenotype can be partially rescued by supplementation with additional nutrients. These phage-resistant bacteria may also be sensitized to membrane-interacting antibiotics. Importantly, LPS mutation led to increased adherence, invasion, and biofilm formation *in vitro* for several of our bacteria, but this phenotype does not result in successful bladder colonization *in vivo*.

Conclusions: While resistance to phages such as ES17 may readily arise in the urinary environment, phage resistance is accompanied by fitness costs rendering UPEC more susceptible to urinary conditions or antibiotics.

Acknowledgements: This work was supported by Baylor College of Medicine's Clinical Translational Research Program T32 to JZ (T32GM136554) and an NIH NIAID U19 award to AM and KP (U19 AI157981).

Uropathogenic *E. coli* resistance to phage ES17 decreases bacterial fitness

Jacob Zulk[1], Marlyd Mejia[1], Vicki Mercado[1], Mallory Ballard[1], Emmaline Heckmann[1], Belkys Sánchez[1], Barbara Trautner[2,3], Anthony Maresso[1], Katy Patras[1]

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[3]Section of Health Services Research, Department of Medicine, Baylor College of Medicine, Houston, Texas, USA

Introduction

Urinary tract infections (UTIs) are amongst the most commonly acquired infections with seven million cases identified in the US each year. UTIs primarily affect women, older adults, individuals with indwelling catheters, and those with underlying medical conditions. The vast majority of UTIs will be caused by uropathogenic *E. coli* (UPEC). A portion of those with UTIs will develop recurrent UTIs requiring additional treatment after the initial infection clears. Current standard of care for those with UTIs includes antibiotics, but as the incidence of antibiotic-resistant UPEC increases, new therapeutics are needed.

Bacteriophage (phage) therapy been suggested as an alternative to antibiotics for several bacterial infections, largely due to its pathogen-specific nature and their wide abundance allowing for rapid isolation. However, to date, phage therapy for treating UTIs has largely been confined to anecdotal, compassionate care use. Importantly, little work has been done to assess what could happen if bacteria become resistant to phage. Groups have investigated the effect of phage-induced mutations in environments such as the blood, however little work has been done in the urinary tract environment. Here, we assess the ability of two UPEC strains, UTI89, a well studied isolate, and DS566, a UPEC isolate from a catheterized patient, to evade phage ES17. We then use *in vitro* growth assays and a mouse model of UTI to assess the effect of these resistance mutations on bacterial fitness. **We hypothesize that resistance to phage will come at the cost of bacterial fitness, a potential therapeutic benefit even in the face of bacterial resistance.**

How do UPEC evade killing by phage ES17?

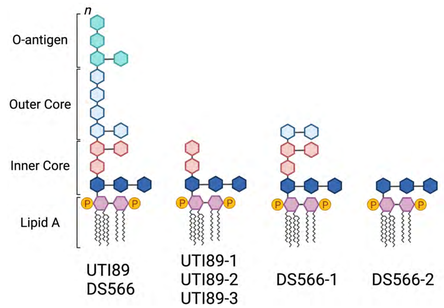
Can these mutations be predicted and exploited?

Isolating Phage Resistant UPEC

Workflow for isolating phage resistant UPEC

ES17-resistant UPEC were isolated by overnight challenge with ES17 in liquid culture before being serially passaged to confirm resistance. Isolated colonies underwent whole genome sequencing to determine mutations.

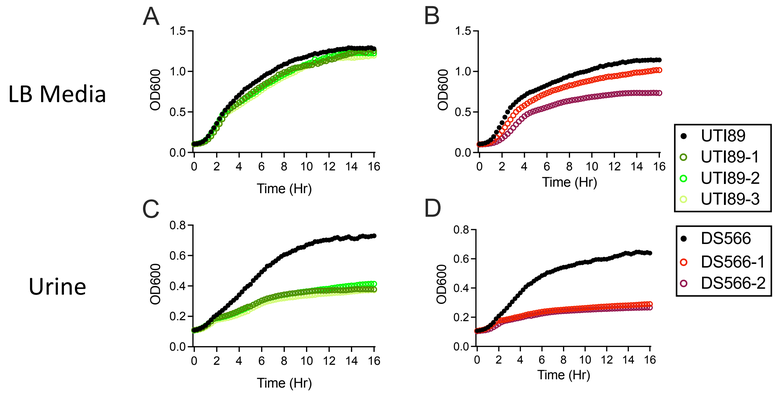
UPEC Evade ES17 by Mutating Lipopolysaccharide (LPS)



Representation of wild-type LPS and expected phage resistant mutant LPS structures

Mutations in LPS were observed for all UPEC isolates resistant to ES17. Individual mutations varied but all are likely to generate UPEC with LPS truncated in the inner or outer core.

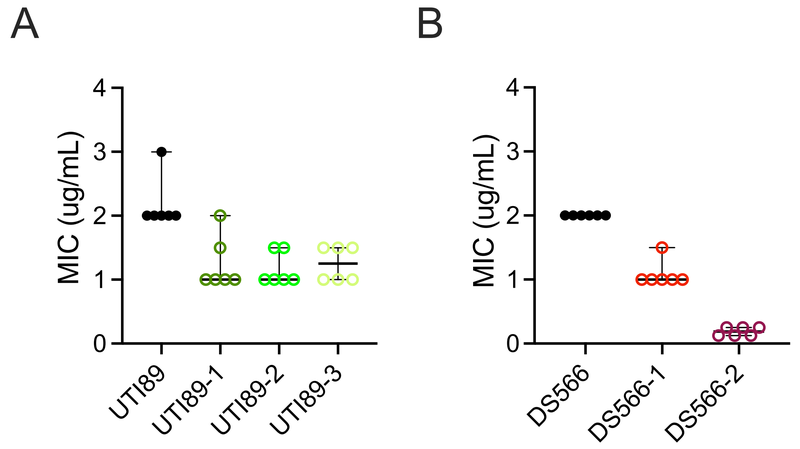
ES17 Resistance Affects UPEC Growth in Urine



ES17 Resistant UPEC grow worse in urine than their parental strains

(A) UTI89 or (B) DS566 and phage resistant mutant growth in LB medium or (C, D) pooled human urine. OD600 measurements taken every 15 minutes for 16 hours. Growth curves are representative of three individual experiments preformed with 3-4 technical replicates.

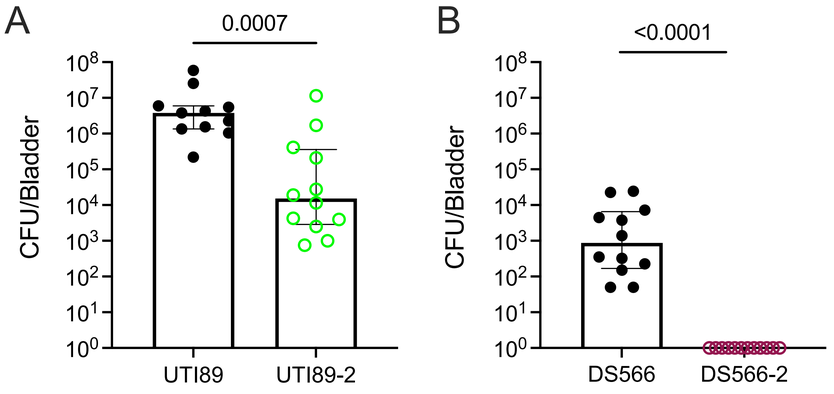
LPS Truncation May Leave UPEC Susceptible to Colistin



UPEC with phage driven LPS mutations are more susceptible to colistin

(A) UTI89 and phage-resistant mutant minimum inhibitory concentration to colistin. (B) DS566 and phage-resistant mutant minimum inhibitory concentration to colistin. Measurements representative of growth after 16 hours of incubation assessed by resazurin measurements. Points are representative of individual experiments performed in duplicate.

LPS Mutant UPEC Poorly Colonize the Bladder



ES17-resistant UPEC have decreased bladder colonization

1 x 10^8 CFU of UPEC strains (A) UTI89 or (B) DS566 or ES17-resistant UPEC were used to transurethrally inoculate C57BL6/J mice. At 24 hours, mice were sacrificed to quantify bladder bacterial burden. Points represent individual mice.

Conclusions

- UPEC with LPS mutations grow more poorly in urine and are poorer at colonizing the murine bladder than their parental strains.
- LPS mutant UPEC with LPS mutations may be more susceptible to membrane-targeting antibiotics.
- Driving mutations that ultimately leave bacteria less fit may be a mechanism for treating UTIs, even if phage resistance emerges.

Future Directions

- Assess the frequency at in which LPS mutations arise to ES17 challenge *in vivo*.
- Investigate the ability of LPS mutant UPEC to invade the bladder and form intracellular reservoirs as well as form biofilms on catheters.
- Study the immune response to LPS mutant UPEC.
- Identify other UPEC factors that are essential for pathogenesis, as well as phage that target them, in order to design better phage therapy cocktails.

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Poster Presentation Talks Day 3

First Name	Last Name	Institution	Poster Title	Day of presentation
Kristen	Curry	RU	*Emu: Species-level Microbial Community Profiling for Full-Length Nanopore 16S Reads*	3
Helen	Ding	Parkland Health & Hospital System	*Institutional Prevalence of Drug-Resistant Pathogens in Community-Acquired Pneumonia*	3
Sam	Erickson	UTH	*Validation of a Disk Elution Synergy Testing Method for Clinical Labs to Determine Susceptibility of Extensively Drug-Resistant (XDR) Gram-negatives to Ceftazidime/Avibactam (CZA) and Aztreonam (ATM) Combination Therapy*	3
Michael	Fischer and Rachel Singer	Texas Department of State Health Services	*Antibiotic Stewardship Policies in Texas Nursing Homes, 2021*	3
Kara	Hood	HMRI	*Topology of LiaF from Enterococcus faecalis Suggests Interaction with LiaX Specific to Enterococcal Species*	3
Tayler	Hunter	UTH	*Investigating the Efficiency of Antibiotic Pocket Irrigants During Breast Reconstruction*	3
Allison	Judge	BCM	*Mapping the Determinants of Antibiotic Catalysis and Substrate Specificity of CTX-M β-lactamases*	3
Jacob	McPherson	UH	*A Clostrioides difficile Capillary Electrophoresis-Based PCR Ribotyping Data Analysis Pipeline, Database and Data Visualization Server*	3
Trevor	Moore	UTH	*Temporal Changes in Antibiotic Susceptibility of Group B Streptococcal Isolates from Young Infants with Invasive Infection: 1970-2020*	3

Poster Presentation Talks Day 3

Mike	Nute* not presenting, Astract and poster included	RU	*A Pan-Genome Analysis of C. difficile Clinical Isolates with Emphasis on Hypervirulent Strain RT027*	3
Jamie	Peña	MDA	*Characterization of Pathogens Recovered from a Dual Blood Culture System Utilized in Patients with Cancer at a Large Academic Cancer Institution*	3
Elizabeth	Sabroske	UTH	*Phenotypic and Genotypic Changes Over Time in Serotype IV GBS Strains*	3
William	Shropshire	MDA	*Diversity of Carbapenem Resistant Mechanisms Distributed Across Enterobacterales Blood Stream Infections at MD Anderson Cancer Center*	3
Rita	Sobral	NOVA University Lisbon	*Skf System, a Promising New Bacteriocin System of Staphylococcus aureus*	3
Xinhao	Song	RU	*Developing Methyl Halide Transferase-Based Gas Reporter as a Novel Growth Quantification Approach for Bacteria in Emulsion Droplets*	3
Benjamin	Strope	MDA	*Molecular Characteristics of Emergent Extended-Spectrum Beta-Lactamase Escherichia Coli Infections at MD Anderson Cancer Center*	3
Truc	Tran	HMRI	*LiaX is Essential for Cell Envelope Adaptation via the LiaFSR System in Enterococcus faecium*	3
M Hassan	Virk	HMRI	*Dynamics Of Colonization and Infection By Multidrug-Resistant Pathogens in Immunocompromised and Critically Ill Patients (DYNAMITE): Preliminary Results*	3

Poster Presentation Talks Day 3

M Hassan	Virk and Rachel Atterstorm	HMRI	*Customizing Populus Plus Lab Inventory Management System for Large Multi Center Observational trial: DYNAMITE*	3
Yizhe	Zhang	RU	*Directed Evolution of Wild Streptomyces towards Antimicrobial Production through Co-Culture with Competitor Pathogens in Microfluidic Droplets*	3

Emu: Species-level Microbial Community Profiling for Full-Length Nanopore 16S Reads

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Background

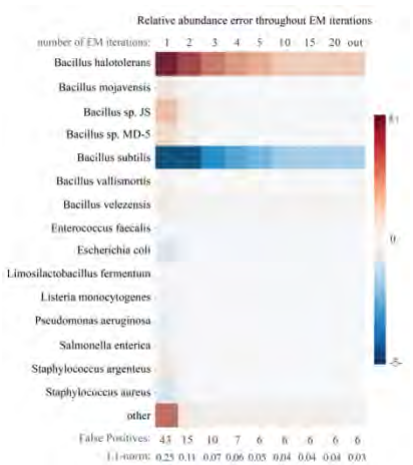
Measurement of microscopic organisms can be useful in a range of applications; the human gut microbiome has shown significant correlations to chronic health conditions, while wastewater microbial information can give insights on pathogens in a community[1,2]. Sequencing the 16S subunit of ribosomal RNA gene of organisms in a community is a standardized approach for establishing microbial profiles through either a short- or long-read sequencing pipeline. Short reads are accepted to be highly accurate[3], but are confined to genus-level precision due to limited read length. Long reads provide potential for species-level precision; however, the only current price-comparable long read sequencing technologies have the formidable obstacle of high error rates[3]. A computational algorithm that can correct sporadic errors produced by long-read sequencers has the potential to generate accurate species-level microbial community profiles.

Hypothesis/Goals

We believe an Expectation-Maximation (EM) approach is suitable for error correction of full-length 16S reads from complex microbial communities. An EM algorithm is an unsupervised machine learning approach to predict unknown variables in incomplete data. We propose an algorithm that tailors this method to accurately predict species-level taxonomy despite unknowns due to sequencing error.

Methods

Our software called Emu is built upon the fundamental knowledge that an unknown sequence is more likely to be of a species that is known to be in the sample rather than introducing a new species. Emu starts by constructing alignments between full-length 16S sequences and a reference database with minimap2[4]. The probability for each alignment is then calculated based on the strength of the alignment and each read is broken down into the likelihood it came from each of the species. The total community composition is then initially calculated directly from these percentages. With each EM iteration, each read's taxonomic assignment probability and the total composition estimation are re-evaluated to give more weight to species with higher probabilities. Once the algorithm detects marginal gains with concurrent iterations, the loop is exited, and final composition estimation is returned.



Results

Emu is especially valuable for distinguishing between genomically-similar species and reducing the number of false positives assumed by the software. On the left is a heatmap demonstrating the reduced error in relative abundance throughout EM iterations of Emu on a known microbial community test set. Here, the furthest left column represents

results generated by a naïve approach and the furthest right column expresses error-corrected results produced by Emu. With concurrent iterations, the error (measured as L1-norm) and number of false positives decreases until the method establishes an equilibrium.

Conclusions

Emu is an active bioconda package available for public use. This is a key contribution in microbiome community research since it supports novel long-read sequencing technologies to increase the feasibility and accuracy in microbiome analysis pipelines. Additionally, the underlying algorithm of Emu can be applied to other data science approaches where information about the data at hand can be leveraged to predict unknowns in the information that would otherwise be impossible to detect.

Acknowledgements

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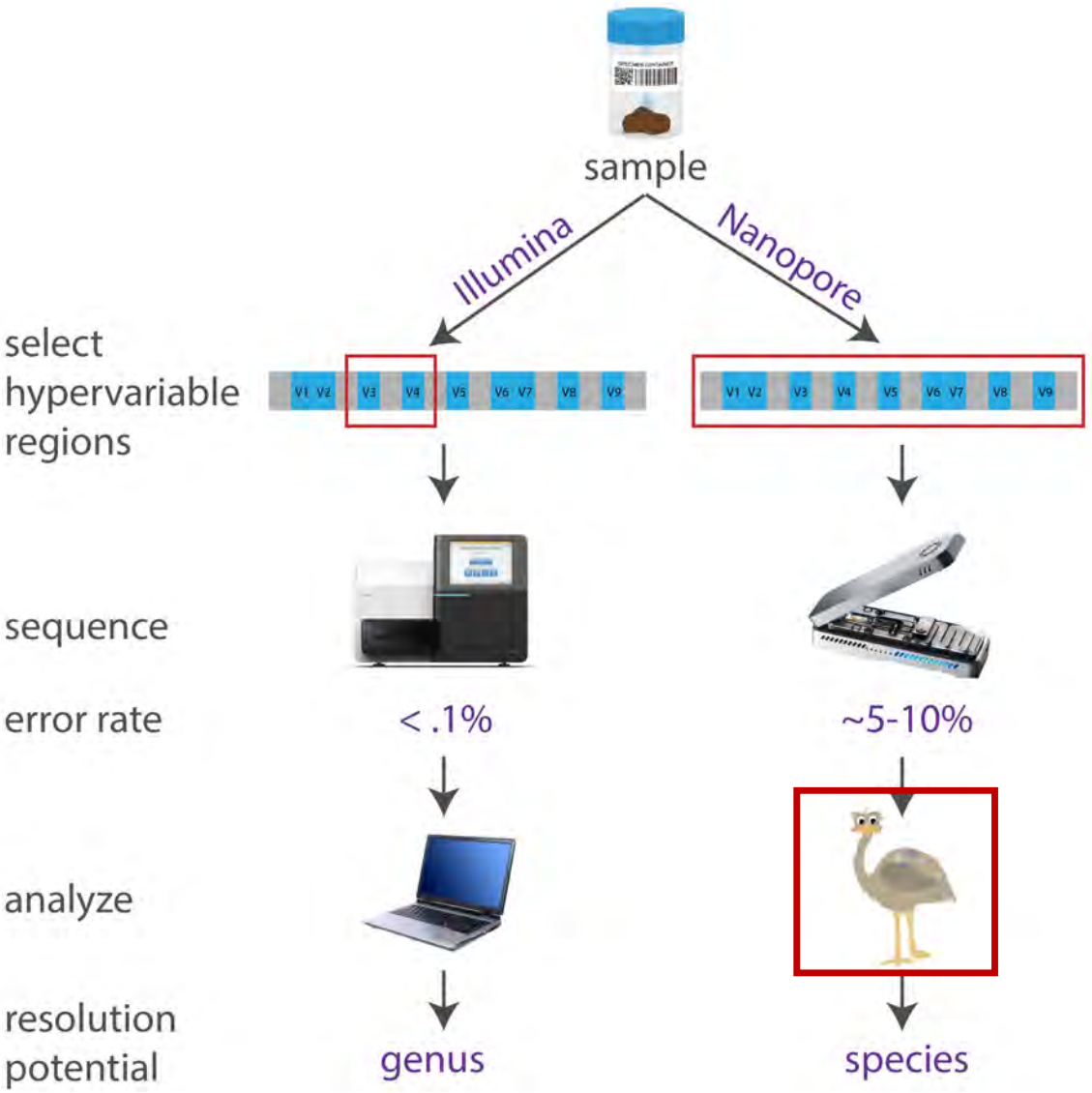
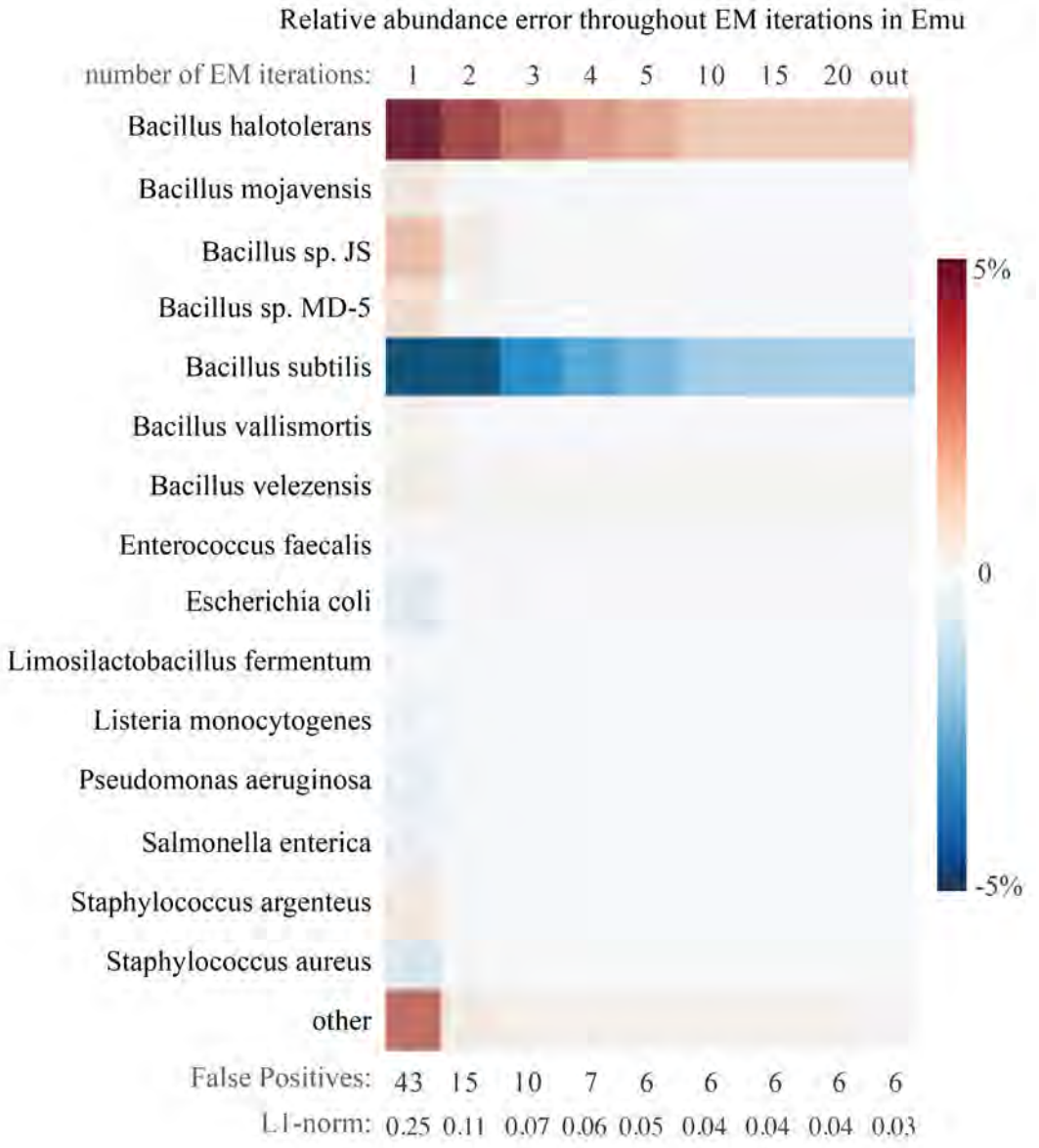
Emu: Species-level microbial community profiling for full-length nanopore 16S reads





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Institutional Prevalence of Drug-Resistant Pathogens in Community-Acquired Pneumonia

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Background: Community-acquired pneumonia (CAP) is a leading cause of hospitalizations and plays a major role in mortality. Over time, the overuse of broad-spectrum antimicrobials has contributed to the emergence of drug-resistant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PsA). The previous Infectious Diseases Society of America (IDSA) definition of healthcare-associated pneumonia (HCAP) led to unnecessary prescribing of broad-spectrum antimicrobials. HCAP risk factors were found to be neither sensitive nor specific for identifying drug-resistant pathogens as a cause of CAP, and use is no longer supported by current guidelines. The 2019 IDSA guidelines for CAP management emphasizes the need for clinician understanding of local epidemiology data to guide selection of appropriate treatment. Currently, local epidemiology data is unknown.

Goals: To determine the prevalence of MRSA and PsA CAP at our institution.

Methods: This was a retrospective observational study of patients admitted to our 870-bed public hospital with a CAP or HCAP diagnosis within 48 hours of admission between March 2016 and March 2021. Patients were excluded if they received a SARS-CoV-2 diagnosis on the same admission, had a history of SARS-CoV-2 requiring intubation, were chronically ventilated, or had an existing tracheostomy or laryngectomy. The primary outcome of prevalence of CAP caused by MRSA or PsA was determined by comparing the number of blood and adequate sputum cultures with MRSA or PsA to total reviewed cases. Secondary outcomes included risk factors associated with CAP caused by MRSA or PsA, utilization of broad-spectrum antimicrobials in CAP, antimicrobial de-escalation within 72 hours if indicated, and CAP treatment duration.

Results: A total of 220 patients were included. MRSA or PsA was isolated in 1.36% of adequate sputum cultures collected (n=3/35) and in no collected blood cultures (n=0/208). The local prevalence of CAP caused by MRSA or PsA among the analyzed sample was 1.36% (n=3/220). MRSA nares screening tests were completed in 10% of cases, 4.5% of which were positive (n=1/22). Due to the small number of patients with CAP caused by MRSA or PsA, risk factors were not analyzed. Broad-spectrum antimicrobials were initially prescribed in 32.3% of cases (n=71/220), with de-escalation within 72 hours occurring in 40.8% of cases (n=29/71). The mean duration of CAP treatment was 7.9 days.

Conclusions: The overall prevalence of CAP caused by MRSA or PsA among admitted patients is low at Parkland Hospital. Increasing the utilization of MRSA nares screening tests for ruling out MRSA CAP will be useful in guiding antimicrobial de-escalation in those receiving broad-spectrum treatment. Future research is needed to identify local risk factors associated with CAP caused by drug-resistant pathogens.

Institutional Prevalence of Drug-Resistant Pathogens in Community-Acquired Pneumonia

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Background

- Community-acquired pneumonia (CAP) is a leading cause of hospitalizations, plays a major role in reported mortality, and incurs significant costs.[1]
- Prior definition of HCAP led to increased prescribing of unnecessary broad-spectrum antimicrobials.
- Treatment regimens involving the unnecessary use of broad-spectrum antimicrobials has contributed to the emergence of drug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PsA).[2]
- HCAP risk factors were neither sensitive nor specific to identify drug-resistant pathogens as a cause of CAP. Use of HCAP risk factors to guide antimicrobial therapy has therefore been removed from the Infectious Diseases Society of America (IDSA) HAP/VAP guidelines and are not supported by the 2019 IDSA CAP guidelines. [3,4]
- The 2019 IDSA guidelines for management of adults with CAP emphasize the need for clinician understanding of local epidemiology data and validated risk factors to guide the selection of appropriate antimicrobial treatment.[4]
- Currently, local epidemiology data is unknown.

Objective

To determine the prevalence of MRSA and PsA CAP and identify risk factors associated with CAP caused by drug-resistant pathogens at our institution.

Methods

- Retrospective observational study
- Conducted at 870-bed public county hospital
- Prevalence was determined by comparing the number of blood and adequate sputum cultures with MRSA or PsA isolates to the total number of CAP cases analyzed.

Inclusion Criteria

- Admitted to Parkland Hospital
- Received a clinical diagnosis of CAP or HCAP within 48 hours of admission between March 2016 and March 2021

Exclusion Criteria

- Diagnosis of SARS-CoV-2 on same admission
- History of SARS-CoV-2 that required intubation
- Chronic ventilation
- Existing tracheostomy or laryngectomy

Results

Table 1. Prevalence of MRSA and PsA Based on Culture Data

Total CAP Cases	Type of Culture Obtained	Cultures Collected	Cultures with Expected CAP Organism Isolated	Cultures with Likely Contaminant Isolated	Cultures with MRSA or PsA Isolated	% Prevalence of MRSA or PsA (Total Cases)
220	Sputum	35	4	1	3	1.36
	Blood	208	6	28	0	0

Table 2. MRSA Nares Screening Test Data

Total CAP Cases	Number of MRSA Nares Screenings Completed	Number of Positive MRSA Nares Screenings	% Positive (Total Cases)
220	22	1	0.45

Table 3. CAP Case Characteristics

Characteristic	Total (N=220)
Age – mean (SD)	
Years	61 (11.2)
Sex – n (%)	
Male	109 (49.5)
Female	111 (50.5)
Race – n (%)	
White	65 (29.5)
Black	155 (70.5)
Comorbid Conditions – n (%)	
Chronic obstructive pulmonary disease	39 (17.7)
Bronchiectasis	3 (1.4)
Cirrhosis	20 (9.1)
Diabetes mellitus, type 2	81 (36.8)
Chronic kidney disease	49 (22.3)
Human immunodeficiency virus	32 (14.5)
Immunocompromised state	30 (13.6)
Social History – n (%)	
Active tobacco use	82 (37.3)
Residence in nursing home or long-term care (LTC) facility	8 (3.6)
Homelessness	19 (8.6)
Residence in congregate care facility or group home	6 (2.7)
Healthcare-Related Factors – n (%)	
Chronic dialysis	15 (6.8)
Hospitalization (within 90 days prior to admission)	73 (33.2)
Receipt of IV antibiotics (within 90 days prior to admission)	40 (18.2)
Previous isolation of MRSA/PsA from sputum culture (within 1 year prior to admission)	2 (0.9)
Previous isolation of MRSA/PsA from any non-sputum culture (within 1 year prior to admission)	2 (0.9)
Previous positive MRSA nares swab (within 1 year prior to admission)	0 (0)
Co-Infections During Same Admission – n (%)	
Influenza	1 (0.5)
Respiratory syncytial virus (RSV)	1 (0.5)

Conclusions

- Overall prevalence of CAP caused by MRSA and PsA is low (<2%) at Parkland Hospital
- Unable to assess risk factors associated with CAP caused by MRSA and PsA due to small sample size

Strengths and Limitations

Strengths

- Results help identify local prevalence of MRSA and PsA as a cause of CAP
- Analyzed frequency of MRSA or PsA relative to the number of all CAP cases as per IDSA recommendations for assessment of prevalence

Limitations

- Descriptive statistics only
- Limited analysis of patients with CAP caused by MRSA and PsA
- Criteria used for classification of severe CAP differed from IDSA definition of severe CAP
- Findings specific to population at Parkland

Future Directions

- Larger sample size
- Identification of locally validated risk factors for CAP caused by MRSA or PsA at Parkland
- Standardization of criteria for severe CAP
- Assessment of treatment appropriateness

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Validation of a Disk Elution Synergy Testing Method for Clinical Labs to Determine Susceptibility of Extensively Drug-Resistant (XDR) Gram-negatives to Ceftazidime/Avibactam (CZA) and Aztreonam (ATM) Combination Therapy

Khan A[1], Erickson SG[2], Egge SL[1], Arias CA[3,4,5], Miller WR[3,4], Bhatti MM[6]

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Background: Carbapenem-resistant *Enterobacteriales* (CRE) and *P.aeruginosa* (CR-PA) producing Metallo-β-lactamases (MBLs) or GES enzymes cause severe nosocomial infections with no defined treatment. CZA-ATM is a potential therapeutic option with activity against these organisms. However, there is no approved, feasible, testing method for clinical labs to assess this combination and guide clinical decision making.

Hypothesis/Goals: We evaluated the performance of an algorithm using β-lactamase identification followed by disk elution testing to determine the combined activity of CZA-ATM against XDR-Gram-negatives.

Methods: We used 114 representative strains of *Enterobacterales* (*Ent,* namely, *E.coli, Klebsiella spp., E.cloacea*), *A. baumanii,* or *P.aeruginosa* (*PA*). All but 9 strains were from the CDC AR bank; 57 isolates with MBLs or GES enzymes (predicted synergy positive) and 57 isolates with other β-lactamases (predicted negative). The NG-Test CARBA 5 (Hardy Diagnostics) was performed prior to susceptibility testing, to detect β-lactamases (KPC, OXA-48-like, VIM, IMP, NDM) using the same inocula. The reference broth microdilution (BMD) method was performed per CLSI guidelines to determine the MIC to ATM or CZA alone, as well as the MIC of ATM in the presence of a stable concentration of CZA (4 µg/ml of avibactam component). Disk elution was performed with one 30 µg disk each of ATM and CZA eluted in 2 ml Mueller Hinton broth (~16 µg/ml final concentration) for 30 min prior to inoculation, and read as presence (resistant) or absence (susceptible) of growth at 16-20 hours.

Results: The overall positive percent agreement (PPA) of the CARBA 5 test was 100% and the negative percent agreement (NPA) was 80.95% (95% CI: 61.95-91.73%). The CARBA 5 yielded 4 false negatives from failing to detect MBLs in *P. aeruginosa* stains, and did not detect GES producers as this β-lactamase is not on the CARBA 5 test panel. The DE method was 98.33% sensitive and 83.64% specific with 1 false negative from a *K. pneumoniae* harboring IMP-4 that was susceptible to the ATM+CZA combination by BMD that the DE called synergy negative (growth). DE yielded 9 false positives from 6 *PA* and 3 *E. coli* isolates harboring MBLs that were resistant to ATM (MIC >64 ug/ml) and CZA with CZA MICs were at the resistant breakpoint and no change in ATM MIC in the presence of CZA.

Conclusions: Overall, the CARBA 5 test was reliable for the accurate detection of MBLs. Based on the performance of the DE method, our proposed algorithm should be used only for *Enterobacterales* that are

resistant to both ATM and CZA and express an MBL. Isolates that meet this criteria should be tested for synergy. Due to the frequent errors, synergy testing by DE is not recommended for CR-PA due to complex resistance profiles.

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Validation of a Disk Elution Synergy Testing Method for Clinical Labs to Determine Susceptibility of Extensively Drug-Resistant (XDR) Gram-negatives to Ceftazidime/Avibactam (CZA) and Aztreonam (ATM) Combination Therapy

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Abstract

Background: Carbapenem-resistant *Enterobacterales* (CRE) and *P.aeruginosa* (CR-PA) producing Metallo-β-lactamases (MBLs) or GES enzymes cause severe nosocomial infections with no defined treatment. CZA-ATM is a potential therapeutic option with activity against these organisms. However, there is no approved, feasible, testing method for clinical labs to assess this combination and guide clinical decision making.

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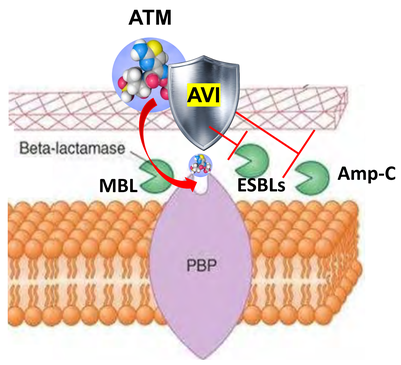
Conclusions: Overall, the CARBA 5 test was reliable for the accurate rapid detection of MBLs. Based on the performance of the DE method, our proposed algorithm should be used only for *Enterobacterales* that are resistant to both ATM and CZA and express a MBL enzyme. Isolates that meet this criteria should be tested for synergy. Due to the frequent errors, synergy testing by DE is not recommended for CR-PA due to complex resistance profiles.

Background

- CRE and CR-PA producing MBLs cause severe nosocomial infections resistant to common therapeutic options[1]. *P. aeruginosa* harboring GES enzymes, serine β-lactamases mimicking MBLs, are an emerging threat[2,3].
- The combination ATM-CZA may have clinical efficacy in infections caused by MBL-producing XDR gram-negatives[2, 4-6].
- CARBA 5 is a rapid lateral flow assay for phenotypic detection of carbapenemase enzymes- KPC, OXA-48 like, NDM, IMP, and VIM.
- **Clinical microbiology labs, however, have no FDA-approved feasible synergy testing method available to guide clinical treatment decisions.**



- MBLs are inefficient at hydrolyzing monobactams like ATM, but frequent plasmid-mediated co-carriage of ESBLs limit its utility for monotherapy.
- Avibactam (AVI) is a diazabicyclooctane inhibitor of class A, C, and OXA-48-like β-lactamase enzymes.
- When CZA and ATM are used in combination, avibactam shields ATM from hydrolysis by class A and C enzymes, and ATM can evade class B MBLs to retain antimicrobial killing activity.

Aim

To evaluate the performance of an algorithm using β-lactamase identification followed by disk elution testing to determine the combined activity of CZA-ATM against XDR-Gram-negatives.

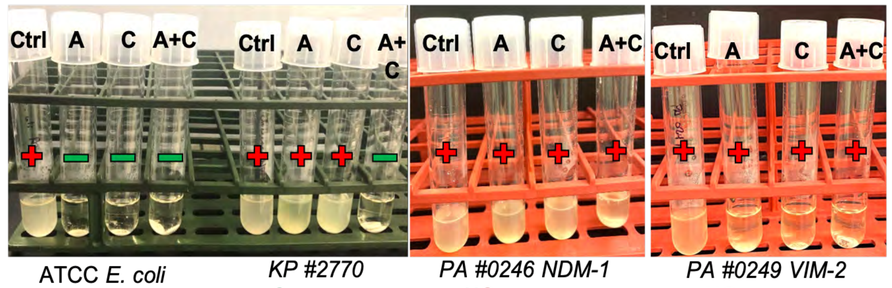
Methods

Strain Selection:
- 114 representative strains of *Enterobacterales*, *A. baumanii*, or *P. aeruginosa*.
- All but 9 strains were from the CDC AR bank.
- 57 isolates with MBLs or GES enzymes (predicted synergy-positive) and 57 isolates with other beta-lactamases (predicted synergy-negative).

Disk Elution:
- One disk each of 30 µg ATM and 30-20 µg CZA eluted in 2 ml MHB (~15 µg/ml ATM and 10 µg/ml AVI final concentration) for 30 min prior to inoculation.
- Read as presence (resistant) or absence (susceptible) of growth at 16-20 hours.
- BMD determined the MIC to ATM or CZA alone, as well as ATM in the presence of a 4 µg/ml stable concentration of avibactam component (designated ATM-CZA).

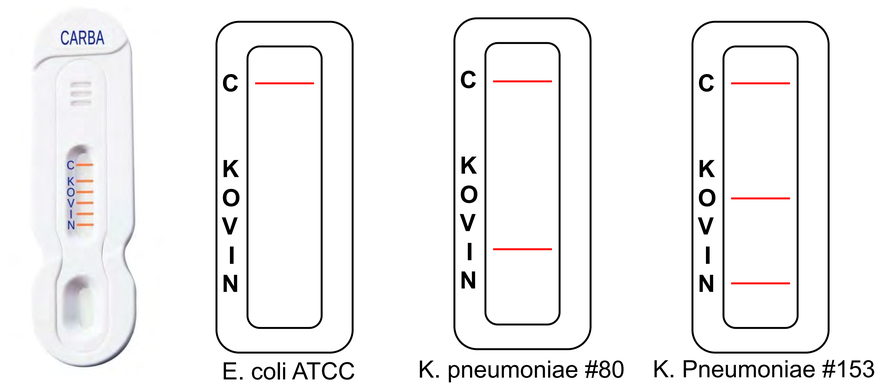
Lateral Flow Assay:
- The NG-Test CARBA 5 was done for each strain using the same inoculum as its respective disk elution test according to the protocol by Hardy Diagnostics.
- Detection of β-lactamases (KPC, OXA-48-like, VIM, IMP, and NDM) from the lateral flow assay were recorded and verified using the genome information from strain banks.

Disk Elution:



ATCC *E. coli* | *KP #2770* **Synergy** | *PA #0246 NDM-1* **NO synergy** | *PA #0249 VIM-2* **NO synergy**

Lateral Flow Assay:



E. coli ATCC | K. pneumoniae #80 | K. Pneumoniae #153

Results

ATM-CZA Sensitive		BMD +	BMD -	
DE	+	59	9	**PPV= 86.76%**
	-	1	46	**NPV= 97.87%**
		Sensitivity= 98.333%	**Specificity = 83.64%**	

There was 1 major error in which a *K. pneumoniae* harboring IMP-4 was susceptible by BMD and called synergy negative by DE. There were 9 very major errors from 6 *PA* and 3 *E. coli* isolates. These were resistant to ATM-CZA by BMD and synergy susceptible by DE.

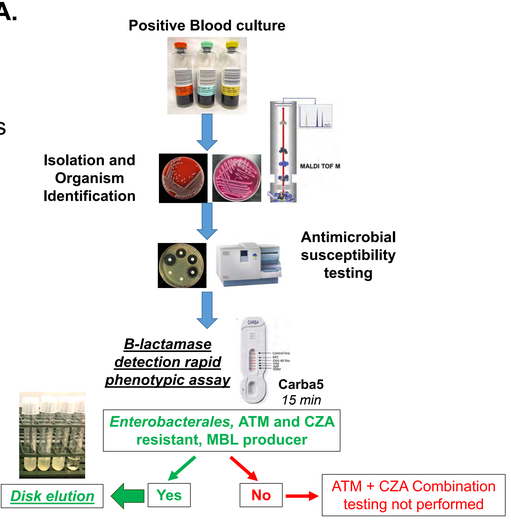
Carbapenemase detection		Known carbapenemase +	Known carbapenemase -	
CARBA 5 Assay	+	93	0	**PPV= 100%**
	-	4	17	**NPV= 80.95%**
		Sensitivity= 95.98%	**Specificity = 100%**	

The CARBA 5 yielded 4 false negatives, all MBLs in *P. aeruginosa* stains, and did not detect GES producers as this β-lactamase is not on the CARBA 5 test panel.

Conclusions

The CARBA 5 test was reliable for the rapid and accurate detection of MBLs. Our proposed algorithm should be used only for *Enterobacterales* expressing a MBL that are resistant to both ATM and CZA.

Due to the frequent errors, combination testing by disk elution is not recommended for CR-PA due to complex resistance profiles.



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Acknowledgements

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Antibiotic Stewardship Policies in Texas Nursing Homes, 2021

Fischer MP[1], Singer R[1], Merengwa EN[1]
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Background: Antibiotic resistance (AR) is an ongoing global public health challenge that pre-dates the current SARS-CoV-2 pandemic. Residents of nursing homes (NH) represent one of the most vulnerable populations to SARS-CoV-2 and AR. With the emergence of SARS-CoV-2, unnecessary antibiotic use has heightened the risk of serious harms to frail and elderly people, such as NH residents. Antibiotic stewardship (AS) refers to a set of commitments and activities designed to optimize the treatment of infections while reducing the adverse events associated with antibiotic use and to promote the appropriate use of antibiotics. Optimizing AS is increasingly important in curtailing the increase and spread of AR during and after the COVID-19 pandemic. Texas DSHS surveyed 116 NH statewide in 2016-2017 and found that 87% had never implemented an AS policy and 93% had not implemented all seven of the CDC's Core Elements of Antibiotic Stewardship for Nursing Homes (CE): leadership, accountability, drug expertise, action, tracking, reporting, and education.

Hypothesis/Goals: An AS policy writing workshop will facilitate the implementation of AS policies in NH, improving the proportion of Texas NH that implement all seven CE. Post-workshop surveys will help to identify perceived barriers NH face in implementing CDC's CE and potential ways the state health department and partners can mitigate those barriers.

Methods: A two-day AS policy writing workshop was held virtually in March 2021 with a convenience sample of NH recruited using state and partner contact lists. The workshop introduced AR and AS in NH and included a 2-question, pre-workshop survey to capture baseline AS policy implementation. Two post-workshop surveys, at 6-weeks and 12-weeks, were conducted to assess the status and barriers to the implementation of AS policies in NH. The unit of analysis for proportion of NH meeting CE was at the facility-level whereas the analysis of reported barriers and needs was at the respondent level. NH were included in analyses if a representative from their facility completed at least one of the post-workshop surveys. For multi-component questions about the implementation of a particular CE, the NH was considered to have met the CE if the survey respondent answered "yes" to any one of the components.

Results: 55 and 79 facilities completed the 6-week and 12-week post-workshop surveys, respectively. 95 unique NH completed at least one post-workshop survey. 69 respondents completed the survey questions specific to barriers and needs to facilitate AS policy implementation in their NH. 54 (56.8%) NH reported meeting all CE post-workshop. The three most frequent barriers to AS cited were lack of time, access to expertise, and resident/family education. The two most helpful resources cited were training for clinical staff and resident education.

Conclusions: Compared to the 7% of Texas NH that reported implementing all seven CE in 2016-2017, 56.8% of the NH in the post-workshop sample reported meeting all CE, indicating a significant improvement from prior years. The most frequently cited barrier to the implementation of AS policy in Texas NH was time, indicating that DSHS can focus improvement efforts on leadership and education as core elements.

Acknowledgements: Surveys were supported by CDC 2019-2024 ELC Cooperative Agreement.

Antibiotic Stewardship Policies in Texas Nursing Homes, 2021.

Michael P. Fischer, MD, MPH & TM, Rachael Singer, PhD, MSPH, a-IPC, Enyinnaya N. Merengwa, MD, DrPH, MHA, MPH

Texas Department of State Health Services, Healthcare Safety Unit, Antibiotic Stewardship Program

TEXAS Health and Human Services | Texas Department of State Health Services

dshs.texas.gov

Title:

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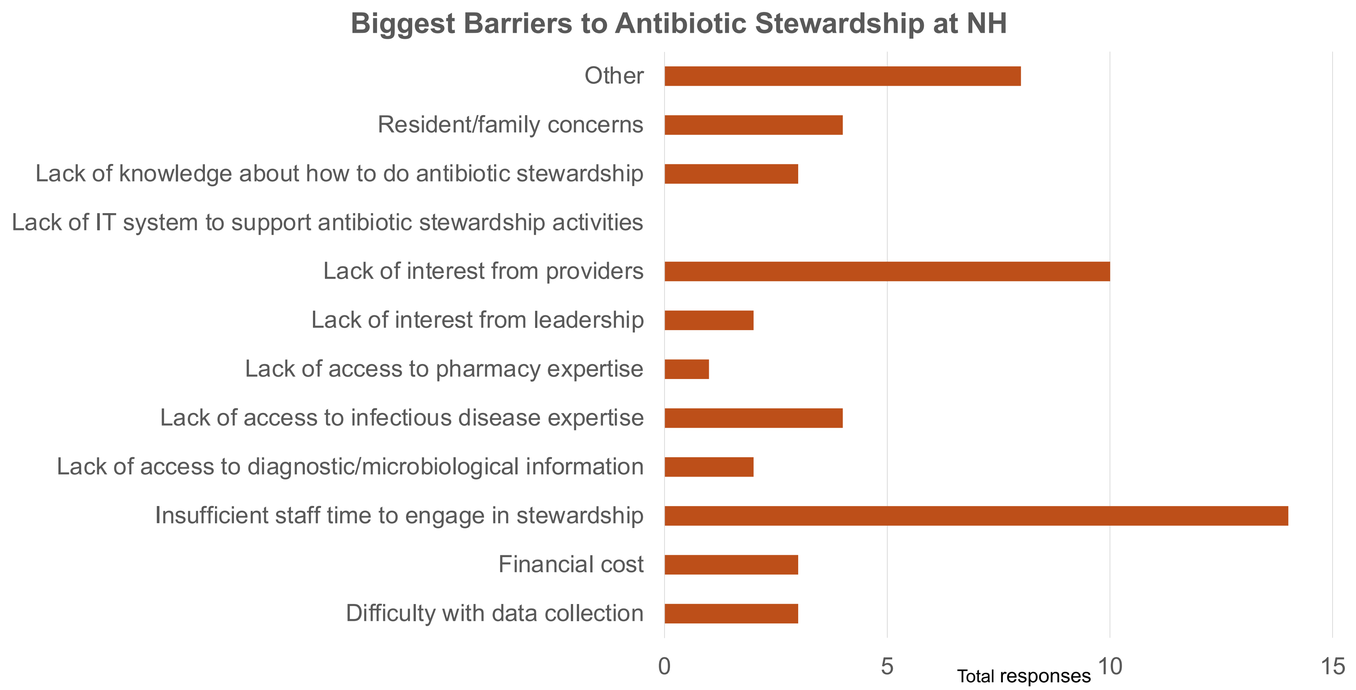

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Acknowledgements:

Surveys were supported by CDC 2019-2024 ELC Cooperative Agreement.

Topology of LiaF from Enterococcus faecalis Suggests Interaction with LiaX Specific to Enterococcal Species

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BACKGROUND: VRE (Vancomycin Resistant Enterococci)-associated infections are becoming more prevalent in healthcare settings and pose a significant therapeutic challenge. The lipopeptide antimicrobial daptomycin is one of the few available options to treat these organisms. Among VRE strains, mutations that confer daptomycin resistance often arise in proteins in the three-component signaling system LiaFSR. The LiaFSR system is conserved across *Firmicutes*, but previous data suggests that, in enterococci, LiaF has a distinct role in system regulation from other species. In *Bacillus subtilis* and *Streptococci* spp, LiaF has been shown to inhibit phosphorylation of the response regulator. In contrast, our data suggest that, in enterococci, LiaF works as an activator of the LiaFSR system and this activation is inhibited by the C-terminal of a novel enterococcal protein designated LiaX.

HYPOTHESIS: We postulate that conformational differences between LiaF from *Enterococcus faecalis* and LiaF from other *Firmicutes* account for the opposing roles in LiaFSR regulation.

METHODS: LiaF protein predictions were determined through the RoseTTAFold algorithm (Robetta online web server, David Baker, University of Washington). Structural comparisons were conducted using the MatchMaker function in UCSF Chimera. *E. faecalis* (Efs) LiaF topology was mapped experimentally using an *in vivo* LacZ alpha-complementation assay wherein the LacZ-alpha fragment was fused to truncations of LiaF in between its predicted transmembrane domains and analyzed through a blue/white colony screen.

RESULTS: Predicted structures of LiaF from Efs, *Enterococcus faecium* (Efm), *Bacillus subtilis* (Bsu), and *Streptococcus pneumoniae* (Spn) exhibited similar domain organization, with 4 N-terminal transmembrane (TM) helices connected to a C-terminal β-sheet domain by a flexible linker of variable length. When analyzed for structural similarity, the C-terminal domains were the most like one another. In contrast, our analysis of the N-terminal domains revealed distinct differences in the orientation of TM helices 2 and 3 in LiaF from Efs and Efm compared to other LiaF proteins. *In vivo* topological mapping of Efs LiaF showed that the N- and C-termini are likely intracellular, but the loop between TM helices 2 and 3, might be extracellular (rather than intracellular, as predicted in Bsu and Spn).

CONCLUSIONS: Our data suggest that the distinct role of LiaF in the regulation of the LiaFSR response may be due to the topological or conformational differences of this protein in the membrane when compared to that of other *Firmicutes*. This structural difference may be related to the possible interactions of LiaF with LiaX, a unique enterococcal protein that functions as a signal transducer and recognizes the presence of daptomycin and antimicrobial peptides.

ACKNOWLEDGMENTS: This work is supported by a training fellowship awarded to KH from the Gulf Coast Consortia, on the Texas Medical Center Training Program in Antimicrobial Resistance (TPAMR), (NIH Grant No. T32AI141349) and NIH R01-AI134637 awarded to CAA.

Topology of LiaF from *Enterococcus faecalis* Suggests Interaction With LiaX Specific to Enterococcal Species

Kara Hood[1,2], Sabona B. Simbassa[3], Yousif Shamoo[4], Cesar A. Arias[1,2]

1. Division of Infectious Diseases, Department of Medicine, Houston Methodist Hospital 2. Center for Infectious Diseases Research, HMRI
3. Department of Microbiology and Molecular Genetics, University of Texas Health Science Center4. Department of Biosciences, Rice University

Background and Hypothesis

- LiaFSR is a three-component signaling system present throughout Firmicutes that responds to exposure to membrane-targeting antibiotics.
- In *Bacillus subtilis* (Bsub) and *Streptococcus pneumoniae* (Spn), LiaF is an inhibitor to system activation
- In *Enterococcus faecalis* (Efs), LiaF has been proposed to be an activator
- Hypothesis: Conformational differences in LiaF between Enterococci and other Firmicutes account for its differing roles in LiaFSR regulation.

Figure 1. Structure of LiaF

Structures of LiaF created with RoseTTAFold (RoBeTTA lab online server). Structure visualization & comparison with Chimera. *Enterococcus faecium* (Efm)

Results: LiaF proteins are organized similarly, with 4 N-terminal TM helices (NTD) and a C-terminal β-sheet domain (CTD). The highest structural conservation is in the CTD.

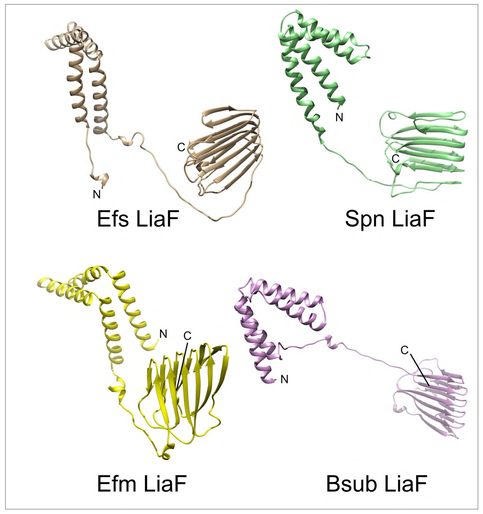
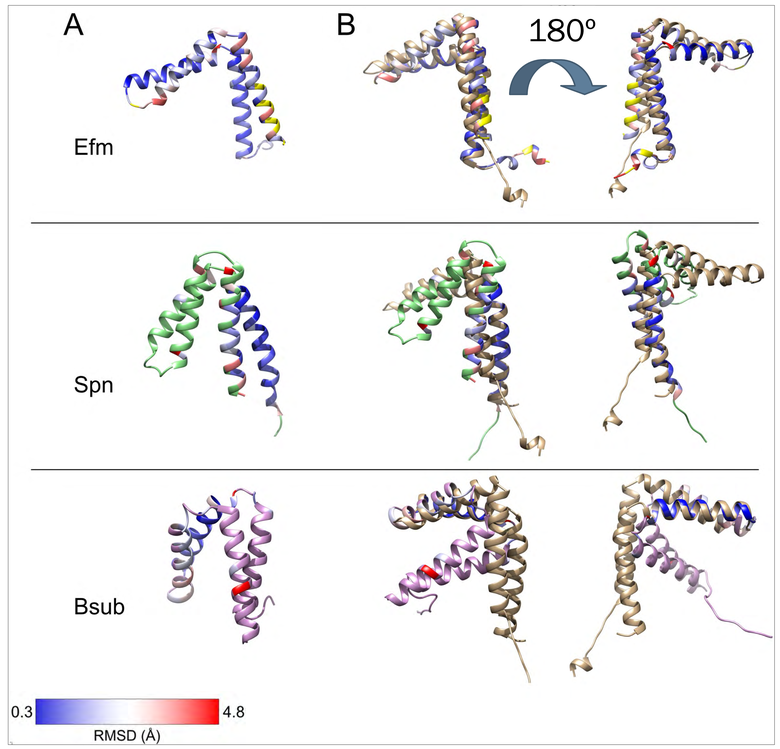


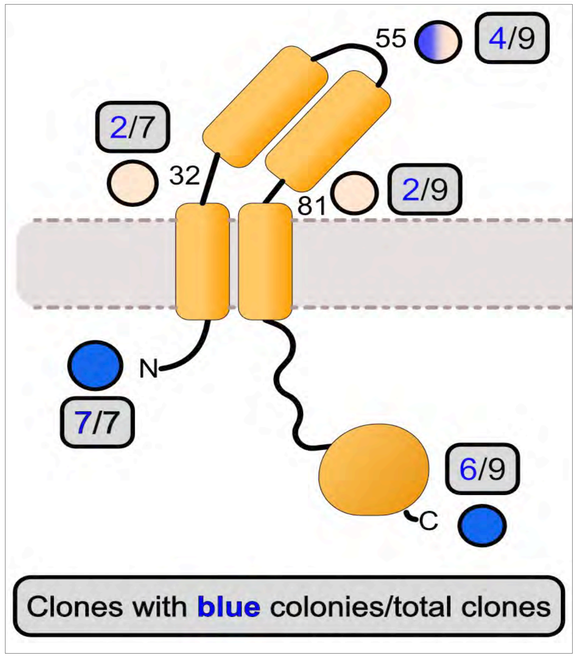
Figure 2. Comparison of LiaF NTDs



Structural comparisons made with MatchMaker and Match→Align functions in Chimera.
(A) LiaF NTD for each species colored according to RMSD when aligned with Efs LiaF NTD.
(B) LiaF NTDs superimposed on Efs LiaF NTD

Results: While Efs and Efm NTDs align well, there are structural differences in the NTDs of Spn and Bsub when aligned with Efs, particularly in the angle between helix pairs 1-4 and 2-3.

Figure 3. Topology mapping of Efs LiaF



LacZ-α complementation and blue/white colony screen to determine intra- or extracellular position of LacZ-α fusions to LiaF truncations (indicated by residue number)

Results: The N- and C-termini of Efs LiaF are both intracellular. The loop between helices 2 and 3 might be extracellular, as suggested by the number of white colonies in $LiaF_{1-55}$--LacZ-α.

Conclusions

Conformational differences in the NTD of LiaF may account for its differential role in regulation. The Enterococcal-specific regulator LiaX can only activate LiaFSR signaling through a functional LiaF, suggesting direct communication. The unique topology of LiaF in Enterococci could be a site of interaction with LiaX and explain why this topology is absent in species like *B. subtilis* and *S. pneumoniae*, which lack true LiaX homologs.

Acknowledgments

KH supported by a training fellowship from the **Gulf Coast Consortia, on the Texas Medical Center Training Program in Antimicrobial Resistance (TPAMR)**, (NIH Grant No. T32AI141349)
Thanks to William R. Miller, M.D., Shivendra Pratap, Ph.D., Diana Panesso-Botero, Ph.D., Truc Tran, Pharm.D., April Nguyen, Ayesha Khan, Ph.D., and Anna Konovalova, Ph.D. for their contributions to this project.

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UCSF Chimera https://www.cgl.ucsf.edu/chimera/

Investigating the Efficiency of Antibiotic Pocket Irrigants During Breast Reconstruction

Hunter TS[1], Duran Ramirez JM[1] , Hanson BM[2] , Bier N[2], Myckatyn TM[3], Walker JN[1]

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2. Department of Epidemiology, School of Public Health, UTHealth
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Background. Ten million women have breast implants worldwide and an additional 300,000 are placed annually in the US for cosmetic and reconstructive purposes. Breast implant-based reconstruction following mastectomy due to cancer has the highest infection rate, occurring in up to 29% of cases, and can result in significant morbidity, including tissue necrosis and disfigurement. Thus, breast implant-associated infections (IAIs) are a highly relevant aspect of women's health. To combat IAIs, major efforts, including the administration of prophylactic antibiotic pocket irrigants, have been implemented, often with conflicting results. Importantly, the most common causes of IAI, staphylococci, are part of the normal human microflora. Increasing studies indicate that it is the staphylococci from within the woman's own microbiome that go on to cause IAI. However, studies investigating the efficacy of prophylactic antibiotic therapy, including triple antibiotic pocket irrigants (TAPI), on infection prevention as well as any effect it may have on the microbiome remain lacking.

Goals. The goals of this study are to understand the composition of the breast microbiome in women i) with cancer; ii) without cancer; and iii) following TAPI prophylaxis.

Methods. To understand the composition of the breast microbiome, we consented and enrolled a total of 16 women (8 with cancer and 8 without cancer) undergoing breast reconstruction at Washington University School of Medicine. Each group was randomized to either the TAPI group or a saline control. The samples collected were skin, breast tissue, tissue expander, capsule, acellular dermal matrix, and drain. We used 16S sequencing to understand the breast microbiome and whether TAPI alters the composition.

Results. These studies indicate that TAPI did not affect the microbial composition of the majority of breast samples compared to controls. The samples with statistically significantly different alpha diversity include tissue expanders from women without cancer that received antibiotics compared to those that received saline and the tissue expanders from women who received TAPI with cancer compared to those without cancer. When testing beta diversity, all tissue expanders with TAPI compared to control displayed statistically significant differences.

Conclusions. The lack of statistically significant results in regard to the majority of the breast samples (cancer and non-cancer) suggests that TAPI may not alter the breast microbiome, but it also calls into question its use as a prophylactic treatment for preventing IAI, since none of the women developed infection. While this study had a small sample size, it highlights the importance of understanding the effect of currently used prophylactic antimicrobial therapies to guide the use of strategies that effectively prevent IAI.

Acknowledgements. A grant from the Plastic Surgery Foundation supported this work.

Investigating the Efficiency of Antibiotic Pocket Irrigants During Breast Reconstruction

Tayler Sydney Hunter[1], Duran Ramirez JM[1] , Hanson BM[2] , Bier N[2], Myckatyn TM[3], Walker JN[1,2]

1. Department of Microbiology and Molecular Genetics, UTHealth
2. Department of Epidemiology, UTHealth
3. Department of Surgery, Washington University School of Medicine

Background

- Breast implants are frequently infected
  - Occur in up to 29% of cases
- The primary method of treatment is implant removal
- Numerous preventive methods in use
  - Administration of prophylactic antibiotics
    - IV
    - Skin scrubs
    - Triple antibiotic pocket irrigants (TAPI)
- Efficacy of TAPI
  - No randomized, controlled clinical studies on infection prevention
  - Unknown effect on the breast microbiome
- The Composition of the Breast Microbiome in Women is understudied
  - Patients with Cancer
  - Patients without Cancer

Goals

To understand the composition of the breast microbiome in women i) with cancer; ii) without cancer; and iii) following TAPI prophylaxis.

Conclusions

The majority of samples had no significant differences. Our data suggests that TAPI may not alter the breast microbiome, but it also calls into question its use as a prophylactic. Future studies are needed to further dissect changes seen on the TE.

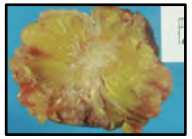
Results

Women Enrolled:

		TAPI		Total
		+	-	
Cancer	+	4	4	8
	-	4	4	8
Total		8	8	16

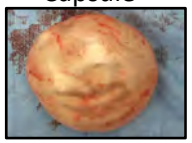
Samples Collected:

Breast Tissue

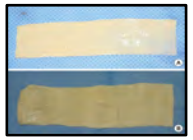


Skin

Capsule



ADM



Tissue Expander

Drain

Figure 1. qPCR of gene counts within samples normalized to 1 g of material

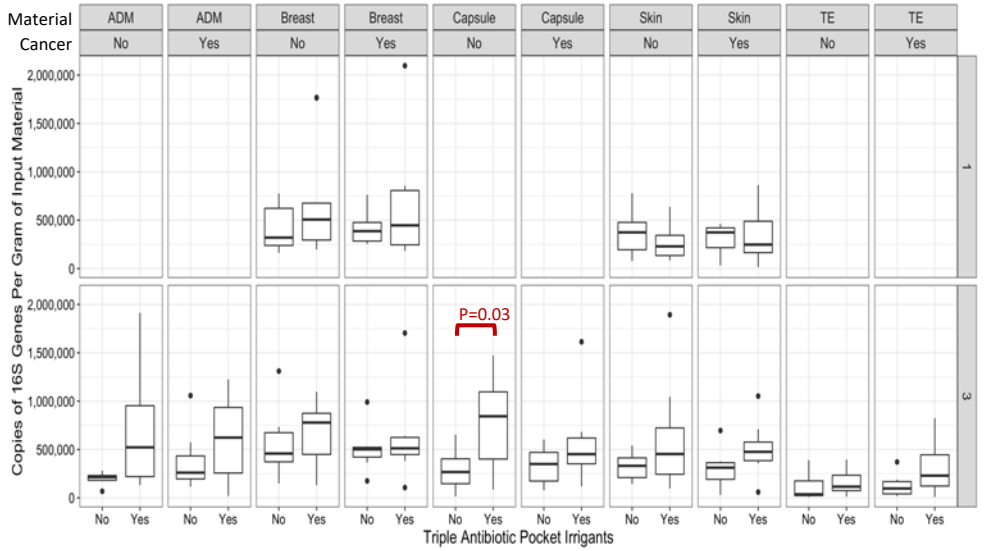


Figure 2. TE microbial clustering in women - cancer

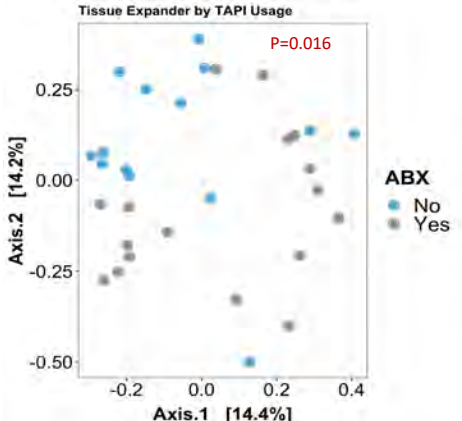
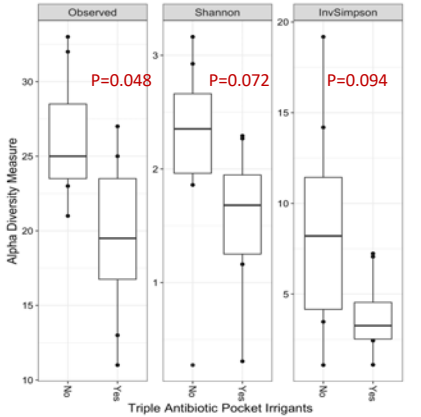


Figure 3. TE microbial diversity in women - cancer



Funding:

THE PLASTIC SURGERY FOUNDATION

UTHealth The University of Texas Health Science Center at Houston

Mapping the Determinants of Antibiotic Catalysis and Substrate Specificity of CTX-M β-lactamases

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Background: Antimicrobial resistance is a growing public health threat, with β-lactamase enzymes causing the largest percentage of resistant clinical infections. β-lactamase enzymes have evolved to hydrolyze even last resort β-lactam antibiotics. This necessitates the rapid design of new antibiotics and β-lactamase inhibitors to provide multiple resources to treat resistant infections. Among β-lactamases, CTX-M enzymes are the most prevalent extended-spectrum enzymes, capable of hydrolyzing a range of β-lactamase antibiotics, including penicillins and the cephalosporin antibiotic, cefotaxime.
Goals: Our goals were to determine the residues within the CTX-M-14 responsible for its extended spectrum antibiotic resistance activity and to determine the basis of potential CTX-M resistance against the extended-spectrum cephalosporin, ceftazidime.
Methods: We constructed individual, codon-randomized libraries for 17 residue positions in the CTX-M-14 active site, including amino acids involved in catalysis and those surrounding them. Each library was introduced into *E. coli* and the population was selected, in separate experiments, for wild-type levels of resistance against two antibiotics that the CTX-M-14 enzyme readily hydrolyzes—ampicillin (a penicillin antibiotic) and cefotaxime (a cephalosporin antibiotic). Additionally, we used the libraries to select for mutants that hydrolyze the cephalosporin ceftazidime, which CTX-M enzymes generally do not hydrolyze, although variants have emerged among clinical isolates that contain mutations leading to ceftazidime resistance. Following selection, mutants were pooled for each library, DNA was extracted, and Illumina NGS sequencing was used to determine the frequency, and therefore the fitness, of each CTX-M-14 mutant relative to wildtype. To confirm sequencing results and further elucidate the mechanisms of antibiotic hydrolysis by mutant enzymes, we determined functional characteristics for select mutants including steady-state enzyme kinetics, minimum inhibitory concentrations, and X-ray crystal structures.
Results: Experiments revealed key catalytic residues required for hydrolysis of all β-lactams tested. Additionally, we found that residues responsible for substrate binding are required for ESBL activity against cefotaxime. Most surprisingly, determination of the ceftazidime sequence requirements revealed that, while some catalytic and substrate binding residues are essential for the limited activity of CTX-M-14 against ceftazidime, key residues within the omega loop (E166, P167, and N170) are not required for hydrolysis.
Conclusions: The results show that CTX-M β-lactamase hydrolysis of cefotaxime is due to a set of active site residues that facilitate substrate binding. Further, these residues are required for cefotaxime but not ampicillin hydrolysis. In addition, our results suggest that, in order for CTX-M enzymes to evolve high levels of resistance against ceftazidime, changes to an omega loop structure at the base of the active site are required. With this information, we can inform target-based drug design and optimize antibiotic-inhibitor cocktails to prevent further evolution of β-lactam resistance by CTX-M enzymes.

Acknowledgements: Research reported in this publication was supported by **R01-032956** from the National Institute of Allergy and Infectious Disease and by **T32-059285** through the University of Texas Health Science Center and the Gulf Coast Consortia.

Mapping the Determinants of Antibiotic Catalysis and Substrate Specificity of CTX-M β-lactamases

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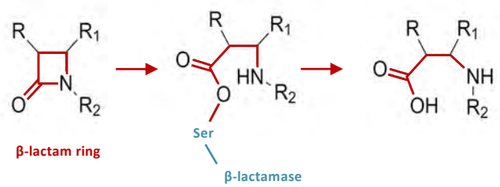
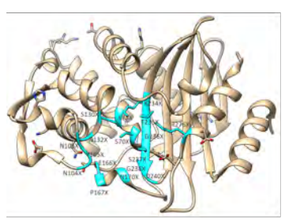
INTRODUCTION

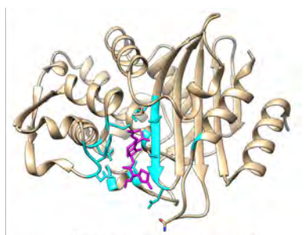
- β-lactamases are the most commonly-found source of antibiotic resistance.
- β-lactamases are plasmid encoded proteins that give gram-negative bacteria resistance by hydrolyzing β-lactam antibiotics using a catalytic serine residue.



- Since their discovery, β-lactamases known as extended spectrum β-lactamases (ESBLs) have evolved to hydrolyze more recently developed β-lactam antibiotics, including penicillins and the cephlosporin cefotaxime (CTX).
- Generally, CTX-M enzymes are not resistant to the cephalosporin ceftazidime, though resistant variants have arisen in the clinic.



CTX-M-14 crystal structure with labeled **active site residues**



CTX-M-14 crystal structure with **cefotaxime** bound in the active site

- CTX-M-14 was chosen as a model.
- 17 residues in and around the active site were chosen to randomize and select for function to determine sequence requirements.

OBJECTIVE

Determine the basis of extended spectrum antibiotic resistance in CTX-M β-lactamase enzymes

EXPERIMENTAL DESIGN

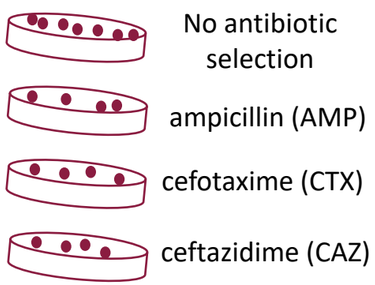
Site-directed mutagenesis

PCR with primer mix containing all possible codons at the chosen site amplifies template to create a randomized library. We created **17 single site randomization libraries**, one for each of the highlighted active site residues.

GGTGAT AAG ACCGGC →

GGTGAT ACG ACCGGC
GGTGAT TGC ACCGGC
GGTGAT TAG ACCGGC
GGTGAT AAG ACCGGC
.....

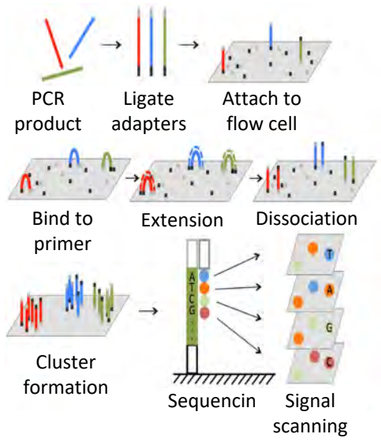
Functional selection



Plasmid extraction

Barcodes added

Amplification and deep

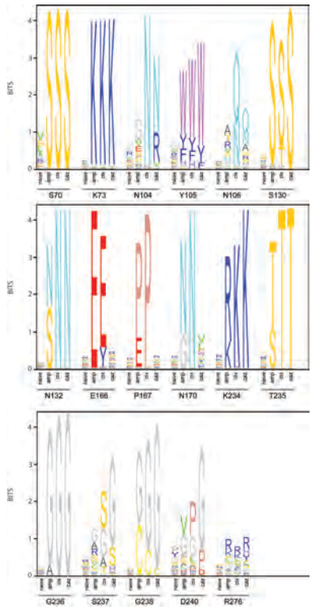


Lu et al, *Next Generation Sequencing in Aquatic Models*, IntechOpen (2015).

Further functional characterization

Well-established techniques including **steady-state enzyme kinetics** and determination of **MICs** (minimum inhibitory concentrations) were used to functionally characterize mutants. This allows us to confirm deep sequencing results as representative of enzyme function.

RESULTS



Key catalytic residues Ser70, Lys73, Ser130, and Gly236 were found to be essential for all β-lactamase activity.

Binding residues essential for extended-spectrum activity Asn132, Pro167, Asn170, Lys234, Thr235, and Gly238 mutations greatly reduce fitness against cefotaxime and ceftazidime.

Ceftazidime selection shows no preference for omega loop residues including Glu166, Pro167, and Asn170, which are highly conserved and have strict sequence requirements against AMP and CTX.

Steady-state kinetic parameters of CTX-M-14^{WT} and variants against ampicillin or cefotaxime.

	AMP			CTX		
	k_{cat}, s^{-1}	K_M, μM	k_{cat}/K_M, $\mu M^{-1}\ s^{-1}$	k_{cat}, s^{-1}	K_M, μM	k_{cat}/K_M, $\mu M^{-1}\ s^{-1}$
WT	55 ± 2	33 ± 4	1.7 ± 0.2	76 ± 3	105 ± 11	0.72 ± 0.08
Y105W	66 ± 6	51 ± 10	1.3 ± 0.28	54 ± 2.5	71 ± 8.6	0.76 ± 0.10
N106Q	27 ± 1	18 ± 2	1.5 ± 0.2	96 ± 3	87 ± 8	1.1 ± 0.1
P167E	72 ± 6	45 ± 7	1.6 ± 0.3	11 ± 0.5	39 ± 5	0.28 ± 0.04
K234R	330 ± 33	1100 ± 170	3.1 ± 0.3	0.2 ± 0.01	11 ± 4	1.8 ± 0.5
T235S	27 ± 1	54 ± 9	0.5 ± 0.09	170 ± 36	2800 ± 760	0.061 ± 0.02
D240P	4.5 ± 0.4	< 5	ND	14 ± 1	15 ± 3	0.93 ± 0.2
D240V	56 ± 3	23 ± 5	2.43 ± 0.5	77 ± 5	45 ± 10	1.7 ± 0.4

CONCLUSIONS

- Kinetic and structural results for CTX-M-14 mutants support that deep sequencing is representative of enzyme function.
- Residues responsible for extended spectrum β-lactamase activity been determined.
- Most notably, we found that ceftazidime activity is independent of otherwise essential omega loop residues, suggesting potential paths to widespread CAZ resistance.

FUNDING SOURCES: NIH R01-AI-2956, NIH T32-GM-120011

A Clostrioides difficile Capillary Electrophoresis-Based PCR Ribotyping Data Analysis Pipeline, Database and Data Visualization Server

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Background: *Clostidioides difficile* is a multidrug-resistant Gram-positive, anerobic, spore-forming bacterium and a leading cause of antibiotic-associated gastrointestinal dysbiosis. Public health monitoring for clonal outbreaks of *C. difficile* infection (CDI) utilizes internationally standardized PCR ribotyping of the variable-length intergenic spacer region (ISR) between the 16S and 23S rRNA genes. Despite these recent methodological advances, there have been limited advancements in the generation of computational pipelines for CDI outbreak monitoring.

Hypothesis/Goals: The purpose of this project is to create a CDI PCR ribotyping server-based pipeline, database, and data-visualization interface.

Methods and Results: Whole genome sequenced CDI isolates of known PCR ribotypes were used to generate .FSA file electropherograms of the 16S-23S rRNA ISR. Data is pushed to an RStudio-based server, where the most probable PCR ribotype is predicted by Bray-Curtis dissimilarity comparison to a manually curated reference database. Confirmatory results are pushed to a biobank-management cloud platform, Nonte (Populus Global Solutions, Fredericton, Canada). Finally, the RStudio pipeline produces real-time visualization of emerging CDI PCR ribotypes, their phylogenetic relatedness, and spatiotemporal routes of transmission.

Conclusions: *C. difficile* PCR ribotyping continues to be the gold standard typing method to monitor outbreaks. The developed pipeline will greatly expand our potential for multi-center collaborations in the future.

Acknowledgements: Populus Global Solutions (Nonte)

A *Clostridioides difficile* Fluorescence PCR Ribotyping Pipeline, Database and Data Visualization Server

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UPDATED ABSTRACT

Background: *Clostridioides difficile* is an anaerobic spore-forming, toxin-producing gram-positive bacillus listed by the CDC as an "urgent threat" hospital-acquired infection.[1] Monitoring for outbreaks of this infectious disease includes PCR amplification of the length-variable intergenic spacer region (ISR) between the 16S and 23S rRNA genes.[2-5] Despite advancements in technologies and internationally standardized protocols, there has been less advancement in the computational realm of a single global repository of ribotyping data, analytical pipeline and contextual metadata. Current efforts with limitations include the online databases "WebRibo", "Ribotyping" and "EnteroBase". An ideal internet-based platform would be a server database, open-sourced pipeline, submitter acknowledged, dual genomic and epidemiologic graphical user interface (GUI).

Specific Aims:
SA1: to Integrate a currently established wet- and dry-lab workflow to an on-premises server. This will utilize current workflows previously described (Alam et al., 2014, Persson et al., 2008; Martinson et al., 2015). Fluorescence PCR Ribotyping amplicons will be injected into a Thermo Fisher SeqStudio Capillary Electrophoresis instrument. Generated Data will be analyzed using a server-adapted branch of https://github.com/nvpinkham/CdiffFragR

SA2: Create a powerful graphical user interface (GUI) for end-user epidemiological and microbiological investigations of CDI. Will seek input from local microbiologists, epidemiologists, antimicrobial stewardship pharmacists and infection control specialists from the Texas Medical Center

SA3: Establish a hybrid-cloud infrastructure using AWS DataSync, File Gateway and Simple Storage Service (S3) to add computational scalability, interoperability and durability, ensuring the longevity of this platform

Next Steps:
Ensure the 10 recommendations for open-source pathogen genomics surveillance put forth by Bedford et al., Nat. Med. 2020

OBJECTIVES

The purpose of this ongoing project is to establish a single hybrid-cloud repository, pipeline and visualization server for CDI Ribotyping community. Currently available recommendations for open-source pathogen surveillance and public health applications will be followed

BACKGROUND

- *Clostridioides difficile* infection (CDI) is a global antibiotic-associated infection that is monitored via PCR ribotyping
- There is a lack of a single unified global CDI PCR Ribotype database, pipeline and visualization platform

METHODS

Prior Workflows

Patient Stool Samples are aliquoted and biobanked for further analyses

↓

Anaerobic Culture Using BHI Broth & CCFA (Alam et al, 2014)

↓

PCR Detection of Toxin Genes (Persson et al, 2008)

↓

Fluorescent PCR Ribotyping of *C. difficile* (Martinson et al, 2015;)

↓

[CDI Ribotyping Server]

Specific Aims

Specific Aim 1: Integrate a currently established wet- and dry-lab workflow to an on-premises sequencer and server

Specific Aim 2: Create a powerful graphical user interface (GUI) for end-user epidemiological context and monitoring for outbreaks of CDI, modeled after NextStrain (*Hadfield et al.*)

Specific Aim 3: Establish a hybrid-cloud infrastructure using AWS DataSync, File Gateway and Simple Storage Service (S3)

Next Steps

Bedford et al.'s 10 recommendations:

1. Consistent data model
2. Strong API
3. Genomic data stewardship guideline
4. Bioinformatics pipelines are fully open-sourced
5. Modular pipelines for data visualization and exploration
6. Reproducibility through standardized datasets
7. Cloud computing to scale
8. A technical workforce
9. Integration with traditional epidemiology
10. Open data sharing best practices with personally identifiable information (PII) in metadata

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Temporal Changes in Antibiotic Susceptibility of Group B Streptococcal Isolates from Young Infants with Invasive Infection: 1970-2020

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Key Words: Infectious disease, antibiotic resistance, *Streptococcus agalactiae*, Group B Streptococcus, macrolides

Background: *Streptococcus agalactiae* (group B *Streptococcus*, GBS) is a leading cause of sepsis and meningitis in neonates and young infants worldwide constituting, a substantial source of morbidity and mortality. GBS is classified based on structural differences in capsular polysaccharides into 10 serotypes (Ia, Ib, II-VII). GBS infant disease, defined as early, late and late-late-onset (EOD/LOD, LLOD), first emerged in the U.S. in the early 1970s as a predominant cause of bacteremia and meningitis. In the early 2000s, GBS screening and intrapartum penicillin prophylaxis of GBS colonized pregnant woman became routine and subsequently reduced EOD by an estimated 80%, but without decreasing LOD or LLOD. Penicillin remains the drug of choice for prophylaxis and treatment of invasive GBS, but use of non-beta-lactam antibiotics, especially macrolides, is common in adults with beta-lactam drug allergy. GBS strains with reduced susceptibility to macrolides, tetracycline, chloramphenicol and vancomycin have been reported. We sought to define the frequency of GBS antibiotic resistance in a collection of invasive GBS isolates from infant EOD, LOD and LLOD spanning from 1970 to the present.
Methods: 2017 GBS isolates from infants with invasive infection from 1970 to 2020 were investigated. Antibiotic susceptibility was assessed phenotypically using 5 antibiotics: penicillin, clindamycin, erythromycin, chloramphenicol and tetracycline. Zones of inhibition surrounding each disk were measured and recorded as either resistant, susceptible or intermediate according to 2019 CLSI breakpoints.
Results: The most common GBS serotypes in our collection were III (n=1112, 55.1%), Ia (n=445, 22%), Ib (n=182, 9%) and II (n=146, 7.2%), all other serotypes accounted for less than 100 isolates each; 6 strains non-typeable. A total of 945 (46.8%) isolates were from infants with EOD, 976 (48.3%) from LOD and 96 (4.75%) from LLOD. All isolates were susceptible to penicillin. Erythromycin and clindamycin resistance increased over time, from 1.1% (3/282) between 1970 and 1979 to 39.8% (145/364) between 2010 and 2020. When compared to the preceding decade, each decade between the 1970s and 2010s showed a significant increase in resistance to both erythromycin and clindamycin, and occurred among all capsular serotypes. Resistance to tetracycline remained high (>80%).
Conclusions: GBS has remained susceptible to penicillin over the past 5 decades. We found increased resistance to both erythromycin and clindamycin over time among all GBS serotypes evaluated. Our results provide insight into the patterns of emerging antibiotic resistance over time among GBS infant disease isolates. GBS resistance to macrolides has increased as their usage has increased in adult as well as pediatric populations.

Temporal Changes in Antibiotic Susceptibility of Neonatal Group B Streptococcus in Houston, Texas

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Introduction

Streptococcus agalactiae (group B *Streptococcus*, GBS) is the leading cause of neonatal sepsis in the United States and constitutes a significant source of morbidity and mortality in neonates worldwide. GBS has been historically classified based on the capsular polysaccharide into 10 serotypes (Ia, Ib,II-IX). GBS infant disease, defined as early-onset disease (EOD; within the first 7 days of life), late-onset disease (LOD; age 7-89 days) and late-late-onset (LLOD; age ≥89 days), first emerged as a predominant cause of bacteremia and meningitis in the early 1970's. Prophylactic treatment with penicillin in pregnant woman with genitourinary tract GBS colonization at admission for deliver is used as prevention for EOD disease and has reduced EOD incidence in the U.S. by ~85%. Penicillin remains the mainstay treatment for prophylaxis and invasive GBS disease. However, the use of second-line, non-β-lactam antibiotics (e.g. macrolides) are common alternative treatments in patients with β-lactam antibiotic allergy. Cases of decreased susceptibility to penicillin, macrolides, tetracycline, chloramphenicol and vancomycin have been reported in GBS. We sought to define the frequency of GBS antibiotic resistance in a collection of neonatal invasive GBS samples spanning from the 1970's to present.

Materials and Methods

- A collection of 2017 neonatal, invasive GBS isolates, spanning 5 decades from Houston, Texas was used for this study (BCJB collection).
- Antibiotic susceptibility was assessed phenotypically using disk diffusion to a total of 5 antibiotics: penicillin (P), clindamycin (CC), erythromycin (E), chloramphenicol (C) and tetracycline (TE).
- Zones of inhibition surrounding each disk were measured and recorded as either resistant, susceptible or intermediate in line with CLSI (2019) breakpoints.

Results

- The most common GBS serotypes in the collection were serotype III (1112), Ia (445), Ib (182) and II (146), all other serotypes had less than 100 isolates and 6 were non-typeable (Figure 1A).
- 945 isolates were EOD, 97 LOD and 90 LLOD. (Figure 1B).
- All of the isolates tested were susceptible to penicillin.
- Resistance to erythromycin and clindamycin has increased over the past 50 years (Figure 2).
- Resistance to tetracycline has remained unchanged with an average of 92% over the last 5 decades (Figure 2).
- Serotype Ia showed a sharp increase in resistance to erythromycin (0% to 60.8%) from 1970 to present, while resistance to clindamycin showed only a slight increase (0 to 3.9%). (Figure 3A)
- Serotype Ib strains also demonstrated an increased resistance to both erythromycin (12.5% to 52.9%), and clindamycin over the period examined (6.3 to 41.2%). (Figure 3B)
- Serotype II showed a sharp increase in resistance to macrolides between the 1990s and 2000s (16.1% to 83.3%), and a similar pattern was also exhibited for clindamycin (Figure 3C).
- Serotype III is the most common serotype to cause invasive GBS in neonates and the most common serotype in our collection (Figure 1A).
- Serotype III strains had a steady increase in resistance to erythromycin and clindamycin, with rates doubling in every successive decade after 1990 for macrolides. (Figure 3D).

Conclusion

GBS remained susceptible to penicillin in our study of isolates from the 1970's to present. Interestingly, we were able to identify increased resistance to both clindamycin and erythromycin over time in all of the serotypes analyzed. When examining the most common serotypes in our collection the same trends in increased resistance to macrolides can be seen. These results are important in that they provide insight into the patterns of antibiotic resistance over time in neonatal GBS. Resistance in these second-line agents has increased as their usage has increased in pediatric populations, such that clinicians should be aware of local resistance patterns.

Figure 1. Neonatal Invasive GBS Sample Distribution by Serotype (A) and Disease Onset (B).

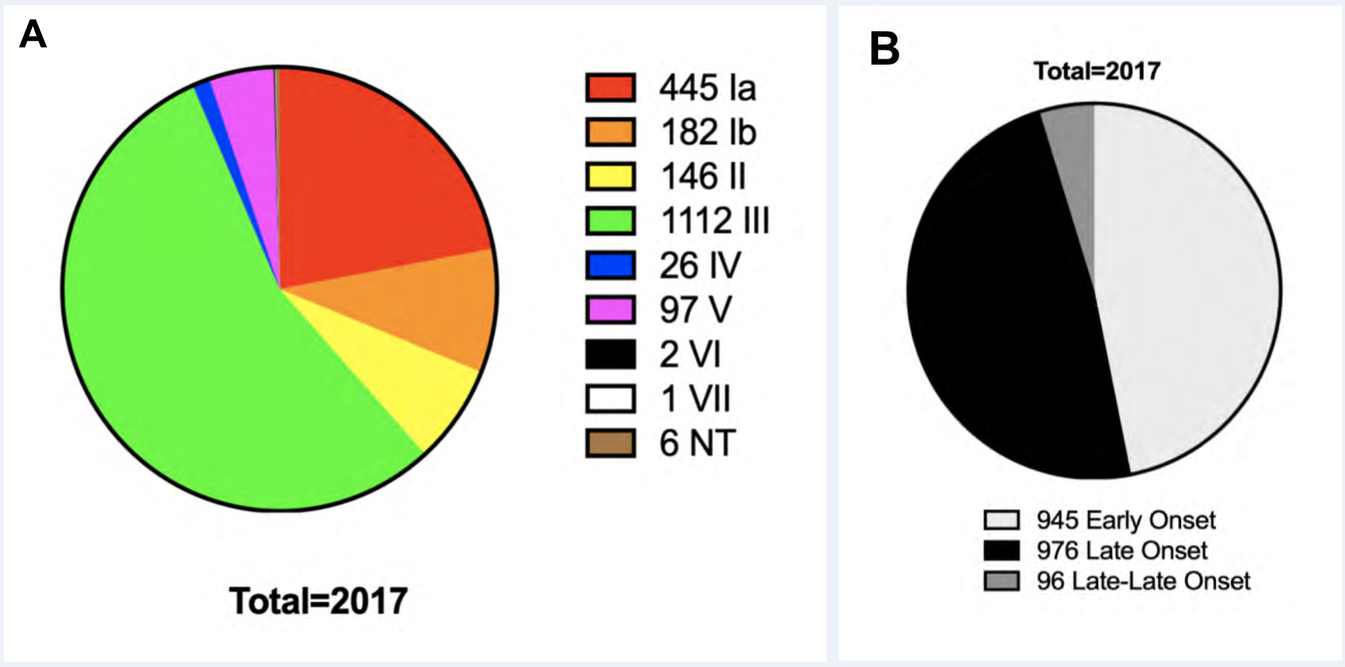
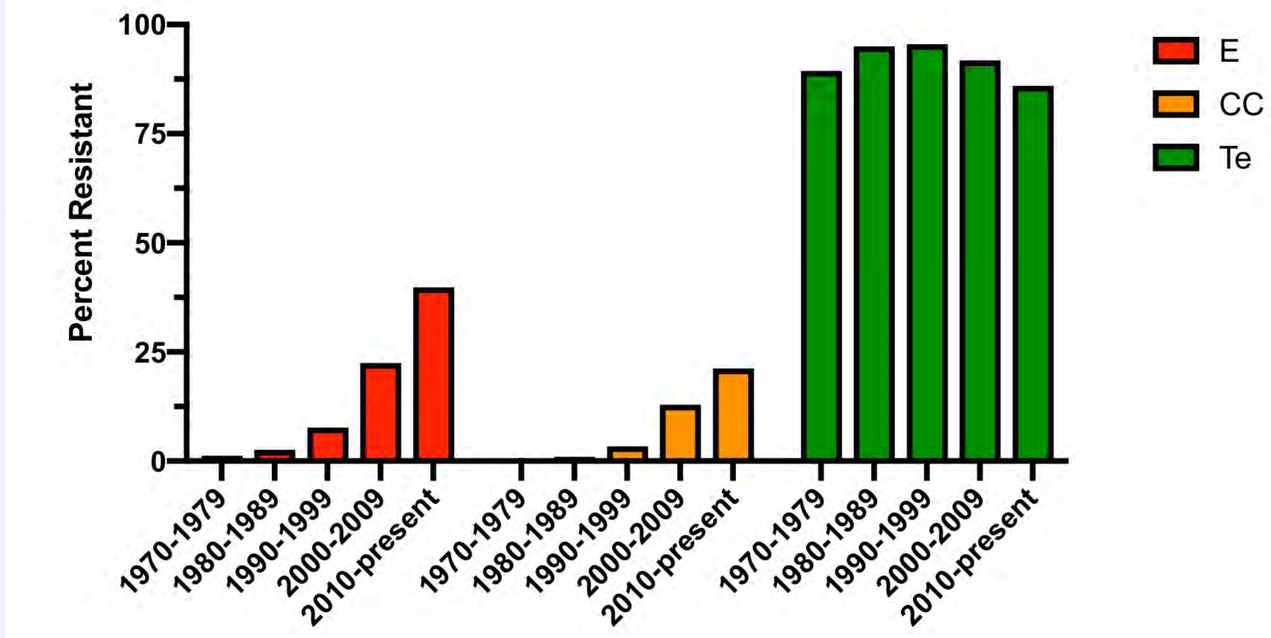
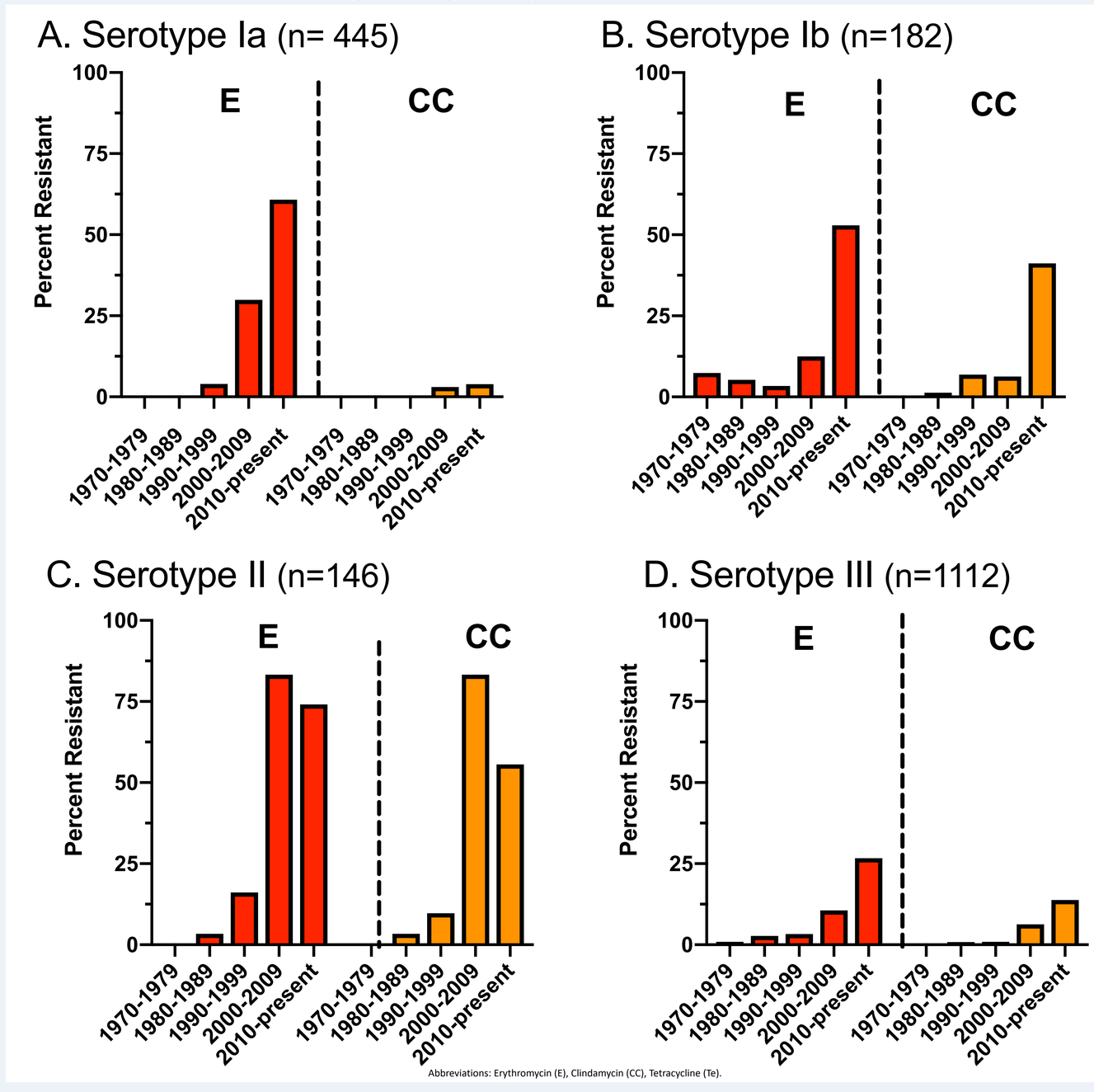


Figure 2. Neonatal Invasive GBS Resistance to Macrolides and Tetracycline from 1970's to present



Abbreviations: Erythromycin (E), Clindamycin (CC), Tetracycline (Te).

Figure 3. Specific differences to Macrolide resistance over time in the the 4 most common GBS serotypes identified in the BCJB collection (1970's to present)



Abbreviations: Erythromycin (E), Clindamycin (CC), Tetracycline (Te).

dA Pan-Genome Analysis of C. difficile Clinical Isolates with Emphasis on Hypervirulent Strain RT027

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Background

Infection by multi-drug resistant *C. difficile* is widespread among hospital patients following treatment with broad spectrum antibiotics, but among the known biodiversity for this pathogen one strain stands out for its association with more severe disease and increased likelihood of recurrence: ribotype 27 (RT027). Studies of this organism have identified several important genetic mutations unique to this strain, in particular in the gene encoding its primary toxin and one of its key regulators, *tcdB* and *tcdC* respectively. But a genome-scale comparison with other strains does not yet exist.

Methods

In this study we present a detailed analysis of whole genome sequencing output from over 800 pathogen isolates obtained clinical samples during a surveillance study across three states and multiple years. This sequencing output was *de novo* assembled using Unicycler, yielding both contigs comprising each individual genome as well as circular contigs containing putative plasmids or phage genomes. The results were combined with reference genomes for multiple common wild-type strains, including RT027, and used as the basis for a multiple genome alignment and pan-genome analysis to understand the diversity of genomes within these clinical samples.

Results

We present several observations from this study as they related to the genome of RT027. First, we show a comparison across the genome to lab strain CD630-Δ*erg*, first cataloging structural changes in the genome and then identifying patterns of significant mutation. We find that RT027 has a genome-wide dissimilarity from the lab strain of just 0.2%, but at a small number of segments in the genome (each spanning multiple genes) the mutation rate is locally much higher. This is given statistical support with a smoothing spline model, and we further show that the assembled genomes of RT027 clinical isolates closely match the reference.

Additionally, we present an analysis of the circular contigs found in the clinical isolates and their genome content. We find a total of 30 contigs that could be identified as orthologous across multiple isolates, with 5 in particular present in at least 2% of the samples. Among these 5 putative plasmids, the genetic diversity ranges from essentially zero to multiple identifiable clades. Finally, since the content of RT027 is of interest *a priori*, we note one additional observation: samples of this ribotype carry circular contigs with a much lower frequency than all other strains ($p < 0.001$).

Conclusions

The genome of *C. difficile* RT027 is materially different than other wild-type strains from clinical isolates. Given this strain's known additional virulence, this comparison may point to previously unknown mechanisms of virulence *C. difficile* as a whole.

Acknowledgements

This work is supported by the NLM Training Program in Biomedical Informatics and Data Science T15-LM007093, Program Director Dr. Lydia Kavraki. Additional support was provided by NIH grants NIAID R01-AI10091401 (Savidge), U01-AI24290 (Savidge) and P01-AI152999 (Arias, et al.).

A Pan-Genome Analysis of *C. difficile* Hypervirulent Strain RT027

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Question: *What makes* C. difficile *RT027 different from other strains?*

Extra-Chromosomal Elements (ECE):

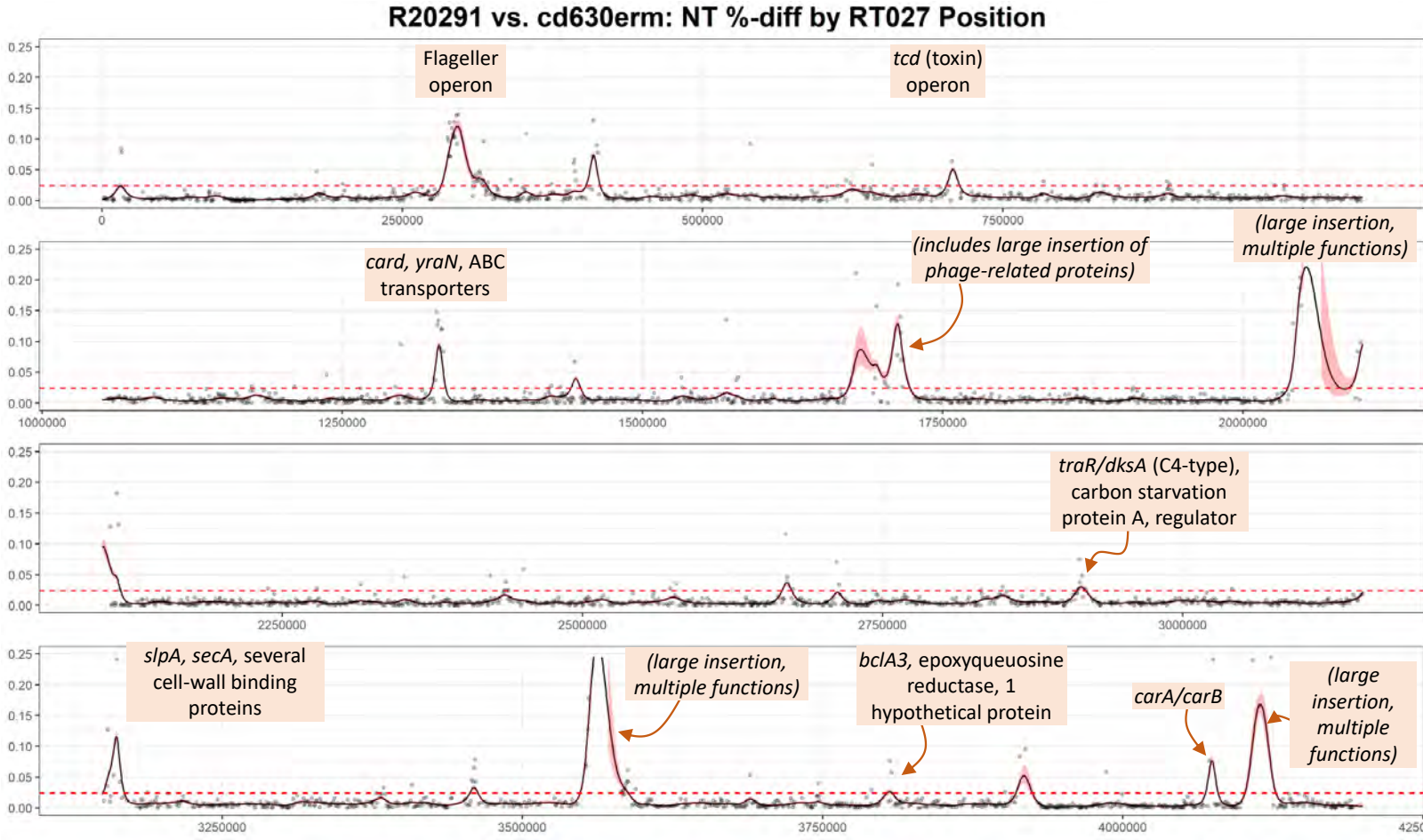
- RT027 carries 0.13 ECE/sample ($n = 187$) vs. 0.44 for all other ($n = 545$)
- RT027 carries plasmid-prophage ϕCD6356 ⟹ RT027 ECE's (sporulation-related)
- Common C. diff plasmids contain ABX *repressor* proteins.
  - *Penicillinase pcd-ECE4/6:* ***present***
  - *tetR, metro resistance:* ***absent***

Conclusions:

- RT027 has several locations with *locally* high mutation rates
- …Near sites of translocation
- RT027 carries few plasmids or phage, with a unique profile

Data:

- RT027 & CD360 annotated reference genomes
- 734 isolate genome assemblies (including circular contigs)



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This work is supported by the NLM Training Program in Biomedical Informatics and Data Science T15-LM007093, Program Director Dr. Lydia Kavraki. Additional support by NIH grants NIAID R01-AI10091401 (Savidge), U01-AI24290 (Savidge) and P01-AI152999 (Arias, et al.).

Characterization of Pathogens Recovered from a Dual Blood Culture System Utilized in Patients with Cancer at a Large Academic Cancer Institution

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Goals: To characterize pathogens recovered in a dual system for blood culture collection (BC) in patients with cancer and bloodstream infections (BSI) at a The University of Texas MD Anderson Cancer Center (MDACC).
Background: Bacteremia in hospitalized patients is associated with high morbidity and mortality, and can lead to sepsis in cancer patients. In addition, BSI in cancer patients can lead to delays in chemotherapy, longer hospitalizations, and increased healthcare costs. The Centers for Disease for Disease Control and Prevention estimates over 1.5 million people in the United States are diagnosed with sepsis annually, causing approximately 250,000 deaths. Initiation of effective antimicrobial therapy targeting the most common pathogens in BSI is critical. Organism recovery is optimized at MDACC by a dual system for BC collection using one aerobic blood culture bottle (Plus Aerobic/F Medium BACTEC™, BD) and one lysis centrifugation tube (Isolator™, Abbott). The advantage of aerobic bottles is continuous, automated pathogen detection through CO_2 production. In contrast, lysis centrifugation tubes (LCT) requires manual reading of plates, but studies suggest superiority in this methodology for isolation of fungi, acid-fast bacillus, and fastidious organisms while also providing quantitative assessments.
Methods: This is a retrospective, observational study of the first 500 positive blood cultures reported in patients with cancer at MDACC in 2018.
Results: Of the first 500 positive BCs, 456 were identified as being mono-microbial, coming from 279 unique patients with primary cancers of solid tumors (43%), leukemia (32%), lymphoma (13%), and stem cell transplant (12%). Gram-positive (GP) organisms were the most common, accounting for 256 (57%) of cultures, followed by 161 (35%) gram-negative rods (GNR), and 38 (8%) fungi. Coagulase-negative staphylococci (CoNS) was the most common GP with 97 (21%) and Enterococcus, non-pneumoniae, Streptococcus, and *S. aureus* each comprising approximately 8% of GP cultures. The most common GNRs were *E. Coli* and *P. aeruginosa*, 78 (48%) and 25 (16%), respectively. Of the fungi, the most common recovered was *C. guilliermondii (*16 of 38 fungal cultures). Further analysis determined that organism recovery from the aerobic bottle alone was 161 (35%), LCT alone was 117 (26%), and the dual-system yielded 177 (39%) positive BCs. Enterococci (12%) and streptococci (14%) were commonly isolated GPs in the aerobic bottle alone, while CoNS (48%) were the most from the LCT BCs alone. Similar recovery of GNRs from both systems was seen with Enterobacterales, accounting for 24% and 16% of aerobic bottle only and LCT only positive, respectively. Recovery of yeasts was higher in aerobic bottles alone compared to LCTs alone, 20 (13%) and 6 (5%), respectively. Four cultures were positive for Fusarium spp.
Conclusion: During the study period, the characterization of positive BCs indicates that the most common GP is CoNS followed by Streptococci and Enterococci. Enterobacterales and *P. aeruginosa* were the most common GNR recovered. Utilization of the LCT BC method yielded a higher rate of recovering GP organisms, specifically CoNS, but the clinical significance of this has not been determined.

Characterization of Pathogens Recovered from a Dual Blood Culture System Utilized in Patients with Cancer at a Large Academic Cancer Institution

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THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

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Goal

Goal: To characterize pathogens recovered in a dual system for blood culture (BC) collection in patients with cancer and blood stream infections (BSI) at MD Anderson Cancer Center (MDACC).

Abstract

Background: Initiation of effective antimicrobial therapy targeting the most common pathogens in BSI is critical. Organism recovery is optimized at MDACC by a dual system for BC collection using one aerobic blood culture bottle (Plus Aerobic/F Medium BACTEC™, BD) and one lysis centrifugation tube (Isolator™, Abbott).

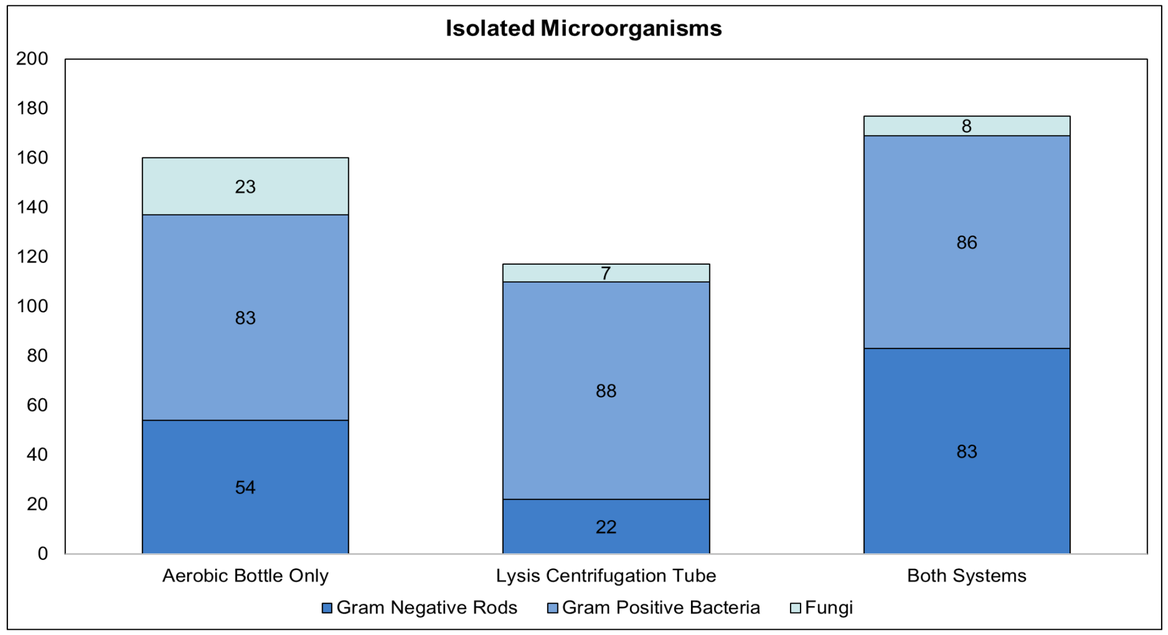
Methods: This is a retrospective, observational study of the first 500 positive blood cultures reported in patients with cancer at MDACC in 2018.

Results: Of the first 500 positive BCs, 456 were identified as being mono-microbial, coming from 279 unique patients with solid tumors (43%) and hematologic tumors (45%); 12% had undergone stem cell transplant. Gram-positive (GP) organisms were the most common, accounting for 256 (57%) of cultures, followed by 161 (35%) gram-negative rods (GNR), and 38 (8%) fungi. Coagulase-negative staphylococci (CoNS) was the most common GP with 97 (21%), *E. Coli* was most common GNR (48%), and *C. guilliermondii* was the most common fungi recovered (16 of 38 fungal cultures). Organism recovery from the aerobic bottle alone was 161 (35%), LCT alone was 117 (26%), and the dual-system yielded 177 (39%) positive BCs. Enterococci (12%) and streptococci (14%) were commonly isolated GPs in the aerobic bottle alone, while CoNS (48%) were the most from the LCT BCs alone. Similar recovery of GNRs from both systems was seen with Enterobacterales. Recovery of yeasts was higher in aerobic bottles alone compared to LCTs alone.

Conclusion: The most common GP found in BC collection was CoNS followed by Streptococci and Enterococci. Enterobacterales and *P. aeruginosa* were the most common GNR recovered. Utilization of the LCT BC method yielded a higher rate of recovering GP organisms, specifically CoNS, but the clinical significance of this has not been determined.

Results

Patient Characteristics	n=279
Age (years), median	57 (6-98)
Sex	
Male	157 (57%)
Female	122 (43%)
Primary Treatment Team	
Solid Tumor	119 (43%)
Hematologic	126 (45%)
Stem Cell Transplant	34 (12%)
Other Characteristics	**n=500**
Type of Blood Draw	
Arterial	4 (8%)
Hemodialysis	8 (1.6%)
Peripheral	160 (32%)
PICC/CVC	196 (39.2%)
Port	62 (12.4%)
Venipuncture	20 (4%)
Other/Not Specified	50 (10%)
Line/Access Removed	157 (31.4%)
Line Replaced	111 (22.2%)



Gram Positive Bacteria	Aerobic Bottle Alone, n=83	LCT Alone, n=88	Both Systems, n=86
Bacillus spp.	1	4	3
Coryneform¥	8	4	6
Enterococcus spp.	19	6	15
Listeria monocytogenes	1	0	1
Staphylococcus aureus	14	6	16
Propionibacterium acnes	0	1	0
Coagulase-Negative Staphylococci	16	56	25
Staphylococci-like§	1	9	0
Streptococcus spp. (non-pneumoniae spp.)	18	2	19
Streptococcus pneumoniae	2	0	1
Streptococcus-like£	3	0	0

¥; *Cellulosimicrobium cellulans*, Corynebacterium spp., *Microbacterium arborescens, Rothia mucilaginosa*
§; Micrococcus spp., Kocuria spp.
£; *Aerococcus viridans, Gemella haemolysans, Granulicatella adiacens*

Gram Negative Bacteria	Aerobic Bottle Alone, n=54	LCT Alone, n=22	Both Systems, n=83
Achromobacter spp.	2	0	1
Aeromonas spp.	0	0	1
Burkholderia spp.	1	0	0
Enterobacterales¥	36	18	62
Pseudomonas aeruginosa	7	2	16
Pseudomonas spp. (non-aeruginosa)	0	0	1
Sphingomonas spp.	2	0	0
Stenotrophomonas maltophilia	1	0	2
Fastidious GNR§	5	2	0

¥; Citrobacter spp., Enterobacter spp., *Escherichia coli*, Klebsiella spp., Proteus spp., Serratia spp.
§; Campylobacter spp. Capnocytophaga spp. *Haemophilus influenzae*, Moraxella spp.

Fungi	Aerobic Bottle Alone, n=23	LCT Alone, n=7	Both Systems, n=8
Candida spp.	21	6	7
Fusarium	2	1	1

Discussion

- Gram positive bacteria were more frequently isolated and more likely to be recovered from the aerobic bottles alone
  - CoNS was the most common GP organism identified
- Gram negative rods were recovered in similar numbers for each system
  - Enterobacterales (*E. coli* and *P. aeruginosa*) were the most common GNRs identified
- Fungi were more often recovered from the aerobic bottle
  - *C. guilliermondii* was the most common fungi recovered
- Clinical relevance is under analysis (i.e. colony count and time to detection)

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Phenotypic and Genotypic Changes Over Time in Serotype IV GBS Strains

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Background *Streptococcus agalactiae* (Group B Streptococcus, GBS) is a leading cause of neonatal sepsis and meningitis worldwide. GBS also causes significant morbidity in adults with co-morbidities and the elderly. GBS is classified by structural differences in capsular polysaccharides into 10 serotypes (Ia, Ib, II-IX). Historically, serotype IV was rarely isolated in the United States, but recent GBS surveillance shows the proportion of serotype IV strains causing early-onset (<7 days of life) and late-onset (7-87 days) disease has increased from 1.3% to 16.2% and 0% to 11%, respectively, from 1999-2019, and for non-pregnant adult disease from 0.7% to 13.4% during the same interval. Penicillin is the drug of choice for treatment and prevention of invasive GBS infection. Macrolides and lincosamides are alternative antibiotics for patients with beta-lactam drug allergy. Concurrent with increasing use of these agents in adults for prophylaxis and treatment, GBS resistance to these second line antibiotics is increasing. In this study, we sought to define the frequency of antimicrobial resistance (AMR) and associated molecular epidemiological features in serotype IV GBS from a collection of strains isolated from infants and adults with invasive disease between 1971 and 2021.

Hypothesis Compared to historical strains, contemporary serotype IV GBS isolates contain differential gene content that contributes to antibiotic resistance and increased prevalence in infant/adult disease.

Methods We used a collection of temporally (1971-2021) and geographically diverse serotype IV GBS isolates causing adult and infant disease for this study (n=223). Antibiotic susceptibility was assessed phenotypically using disk diffusion (penicillin, chloramphenicol, tetracycline, clindamycin, and erythromycin) and CLSI 2021 criteria. Further genetic characterization of strains was performed using whole genome sequencing (WGS).

Results Of the 223 isolates, 30 were infant-associated and 193 were derived from adult disease. Strains isolated before 2000 (n=14) had no resistance to erythromycin or clindamycin but all were resistant to tetracycline. We observed a steady increase in the frequency of erythromycin and clindamycin resistance over time (23% in 2001-2005 to 63% after 2016). However, tetracycline resistance was more variable and in contrast to previous reports in GBS never exceeded 60% among strains isolated after 2000. Strain WGS (n=218) and sequence typing revealed 2 major clonal complexes (CCs): CC452 (35.3%, 77/218) and CC459 (48.2%, 105/218). However, prior to 2000, 75% (8/12) strains were CC1 and no CC452 or CC459 strains were identified. CC452 strains were infrequently associated with antimicrobial resistance or resistance gene presence, with only 13% (10/77) harboring any *tet* allele and 3.9% (3/77) with any gene conferring macrolide resistance. In contrast, CC459 strains showed high frequency of *tet(M)* (82.9%, 87/105) and *erm(A)* (100%, 105/105). Interestingly, 88.6% (93/105) of *erm(A)*-positive CC459 strains showed constitutive resistance to clindamycin from conserved mutations in the upstream *erm(A)* leader peptide.

Conclusions Currently circulating serotype IV GBS CC452 and CC459 strains likely emerged after 2000 and are frequently associated with tetracycline and macrolide resistance. However, this is primarily attributable to CC459 strains. The contribution of antimicrobial resistance to CC459 emergence and forces contributing to the co-circulating but largely antimicrobial susceptible CC452 remain to be explored.

Phenotypic and Genotypic Changes Over Time in Serotype IV GBS Strains

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UTHealth The University of Texas Health Science Center at Houston

Introduction

Streptococcus agalactiae (Group B Streptococcus, GBS) is a leading cause of neonatal sepsis and meningitis worldwide. GBS also causes significant morbidity in adults with co-morbidities and the elderly. GBS is classified by structural differences in capsular polysaccharides into 10 serotypes (Ia, Ib, II-IX). Historically, serotype IV was rarely isolated in the United States, but recent GBS surveillance shows the proportion of serotype IV strains causing early-onset (<7 days of life) and late-onset (7-87 days) disease has increased from 1.3% to 16.2% and 0% to 11%, respectively, from 1999-2019, and for non-pregnant adult disease from 0.7% to 13.4% during the same interval. Penicillin is the drug of choice for treatment and prevention of invasive GBS infection. Macrolides and lincosamides are alternative antibiotics for patients with beta-lactam drug allergy. Concurrent with increasing use of these agents in adults for prophylaxis and treatment, GBS resistance to these second line antibiotics is increasing. In this study, we sought to define the frequency of antimicrobial resistance (AMR) and associated molecular epidemiological features in serotype IV GBS from a collection of strains isolated from infants and adults with invasive disease between 1971 and 2021.

Hypothesis:

Compared to historical strains, contemporary serotype IV GBS isolates contain differential gene content that contributes to antibiotic resistance and increased prevalence in infant/adult disease.

Materials and Methods

- **223 Serotype IV GBS strains**
- **Strains from 1971 - 2021**
- **MLST to elucidate the ST distribution** using Illumina MiSeq short-read sequence data**.**
- **Antimicrobial susceptibility testing of GBS strains** (disc diffusion susceptibility testing).
- **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.
- **Resistance Gene Content** using both short-read sequencing and de novo assembly information.

Conclusions

- CC452 and CC459 strains likely emerged after 2000
- Contemporary serotype IV GBS strains are frequently associated with tetracycline and macrolide resistance, primarily attributable to CC459 strains

Future Directions

- More thorough analysis to understand mechanism behind constitutive resistance to clindamycin.
- Additional phylogenetic analysis to understand why and how CC452 and CC459 emerged.
- Animal models of GBS disease to see if CC452 and CC459 are more capable of causing human disease than other strains of serotype IV GBS.

Results

Figure 1: Clonal complex by years

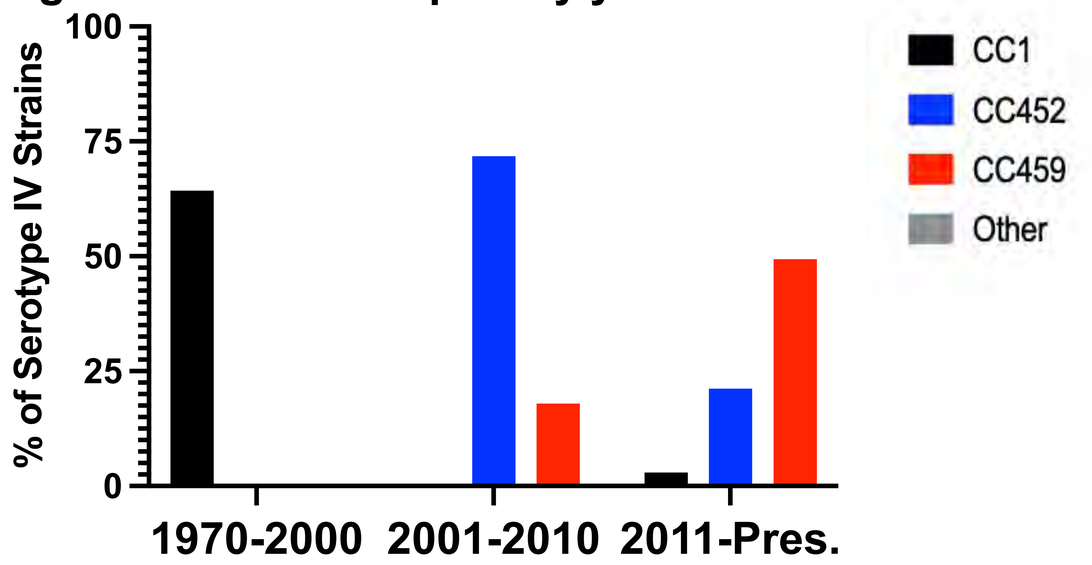


Figure 2: Antibiotic resistance by clonal complex

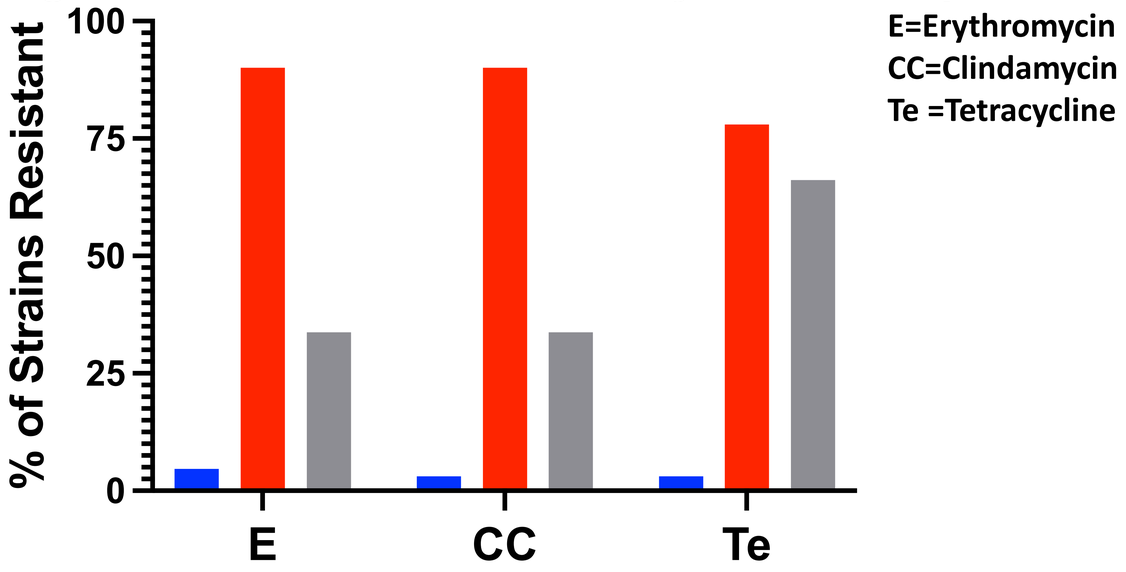
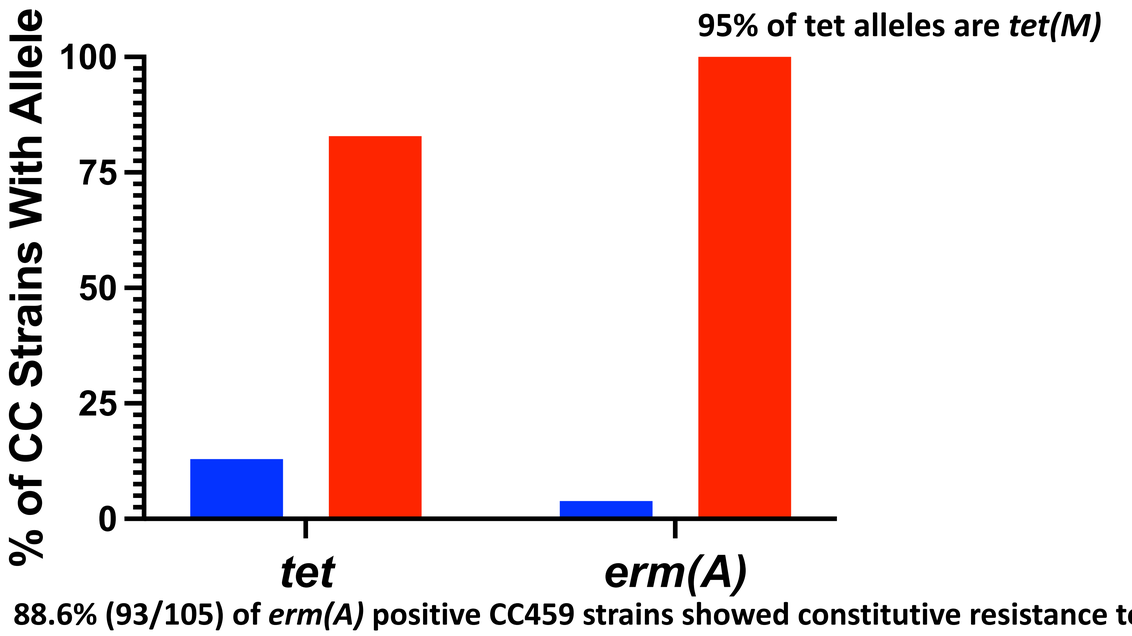


Figure 3: Clonal complex strains with resistance alleles



✓ **88.6% (93/105) of *erm(A)* positive CC459 strains showed constitutive resistance to clindamycin from conserved mutations in the upstream *erm(A)* leader peptide.**

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Diversity of Carbapenem Resistant Mechanisms Distributed Across Enterobacterales Blood Stream Infections at MD Anderson Cancer Center

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Background: Carbapenem resistant *Enterobacterales* (CRE) infections are among the most challenging public health threats. Although there have been recent multi-center CRE surveillance studies, there are limited data regarding CRE epidemiology in immunocompromised patients. Furthermore, there is a gap in knowledge regarding the complex mechanisms underlying the CRE phenotype in absence of a carbapenemase, *i.e.*, non-carbapenemase-producing CRE (non-CP-CRE). Long-read sequencing can elucidate the mobile genetic elements that may contribute to these complicated mechanisms such as antimicrobial resistance (AMR) encoding gene amplification.

Hypothesis/Goals: Characterize CRE isolates causing bacteremia within a four-year time frame at the MD Anderson Cancer Center (MDACC) in Houston, TX.

Methods: Unique Enterobacterial bacteremia episodes were abstracted from the Epic electronic medical record system. Carbapenem resistance was defined based on CLSI M100 (2018) definitions. Strains which tested as non-susceptible to one or more carbapenems and were present in our Microbe Bank collection of stored bloodstream isolates had ertapenem (ETP) MICs confirmed through ETEST® (bioMeriux, Inc). Isolates with confirmed ETP non-susceptible or resistant phenotypes underwent WGS using both short-read (Illumina) and long-read (Oxford Nanopore Technologies) platforms. Isolates with intermediate resistance to ETP, meropenem, or imipenem were categorized as carbapenem non-susceptible *Enterobacterales* (CNSE). Isolates with carbapenem resistance defined by CLSI M100 were categorized as either carbapenemase-producing *Enterobacterales* (CPE) or non-CP-CRE based on AMR gene detection with whole genome sequencing (WGS) data. A custom bioinformatics pipeline was used for AMR gene detection, multi-locus sequencing typing, and phylogenetic analysis.

Results: There were 1632 unique *Enterobacterales* blood stream infections (BSIs) identified from July 2016 to June 2020. A total of 7.0% (114/1632; 95% Confidence Interval (CI): 5.9, 8.3%) were CNSE or CRE with 5.2% (85/1632; 95% CI: 4.2, 6.4%) being identified as CRE by the clinical microbiology

laboratory. A higher proportion of *K. pneumoniae* BSIs (8.6%; 29/339) were carbapenem resistant as compared to *E. coli* (4.1%; 38/939) (Fisher's exact p value = 0.01). Of the 79/114 (69.3%) isolates that met our WGS criteria, the majority were non-CP-CRE (41/79; 51.9%) while 25.3% were CNSE (20/79) and 22.8% were CPE (18/79). There were statistically significant increases (Wilcoxon Test; p-value < 0.05) in median copy numbers of extended-spectrum beta-lactamase encoding genes (ESBLs) for both non-CP-CR *E. coli* (5.2 copies; n= 19) and *K. pneumoniae* (3.8 copies, n = 18). The primary drivers of gene amplification were IS*26* and IS*Ecp1* transposons harboring $bla_{\text{CTX-M}}$ variants with both plasmid and chromosomal contexts. All non-CP-CR *E. coli* and *K. pneumoniae* had predicted reduced expression of at least one outer membrane porin (*i.e. ompC/ompF* or *ompK36/ompK35*) encoding gene. Furthermore, both *E. coli* (CPE ETP MIC = 18.0 μg/mL; non-CP-CRE ETP MIC = 19.7 μg/mL) and *K. pneumoniae* (CPE ETP MIC = 24.0 μg/mL; non-CP-CRE ETP MIC = 25.4 μg/mL) had similar ETP MICs independent of their respective CRE category.

Conclusions: The majority of CRE bacteremia isolates sequenced during our study time frame were non-CP-CRE with transposon mediated amplifications of ESBL encoding genes and porin disruption. The use of long-read WGS facilitates understanding of the mechanisms driving the non-CP-CRE phenotype.

Diversity of Carbapenem Resistant Mechanisms Distributed Across *Enterobacterales* Blood Stream Infections at MD Anderson Cancer Center

Shropshire WC[1], Konovalova A[2], McDaneld P[3], Sahasrabhojane P[1], Greenberg D[4,5], Kim J[6], Zhan X[6], Aitken S[7], Bhatti M[8], Hanson BM[9], Arias CA[10], Shelburne SA[1,11*]

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INTRODUCTION

Carbapenem resistant *Enterobacterales* (CRE) infections are among the most challenging complications for immunocompromised patient populations. Although there have been recent multi-center CRE surveillance studies, there are limited data regarding CRE epidemiology in immunocompromised patients, particularly for bloodstream isolates. Additionally, the lack of long-read sequencing for CRE isolates means there are limited understanding of the complex mechanisms underlying the CRE phenotype in absence of a carbapenemase (i.e., non-CP-CRE).

METHODS

Unique Enterobacterial bacteremia episodes were abstracted from the Epic electronic medical record system. Strains which had tested as non-susceptible to one or more carbapenems and were present in our Microbe Bank collection of stored bloodstream isolates had ertapenem (ETP) MICs confirmed through ETest (bioMeriux, Inc). Carbapenem resistance was defined based on CLSI M100 (2018) definitions. Isolates with intermediate resistance to ETP, meropenem, or imipenem were defined as carbapenem non-susceptible *Enterobacterales* (CNSE). Isolates with CLSI defined carbapenem resistance were defined as either carbapenemase-producing *Enterobacterales* (CPE) or non-carbapenemase-producing CRE (non-CP-CRE) based on carbapenemase detection using whole genome sequencing (WGS) and MALDI-TOF results respectively. Isolates with confirmed ertapenem non-susceptible phenotype were whole genome sequenced using both short-read (Illumina) and long-read (Oxford Nanopore Technologies) platforms. Computational and bioinformatic analysis was performed on a UTHealth high performance cluster server. Statistical analysis was performed using R-v4.0.4.

RESULTS

Table 1: Molecular Epidemiology of Carbapenem Non-Susceptible Enterobacterales Blood Stream Infections July 2016 – June 2020

	CNSE (n = 20; 25.3%)	Non-CP-CRE (n = 41; 51.9%)	CPE (n = 18; 22.8%)	p-value
Enterobacterales Species (n;%)				0.02
Escherichia coli (n = 37; 46.8%)	12 (32.4)	19 (51.4)	6 (16.2)	
Klebsiella pneumoniae (n = 28; 35.4%)	2 (7.1)	18 (64.3)	8 (28.6)	
Enterobacter spp. (n = 8; 10.1%)	5 (62.5)	2 (25.0)	1 (12.5)	
Other (n = 6; 7.6%)	1 (16.7)	2 (33.3)	3 (50.0)	
Source of bacteremia (n;%)				NS
GI	6 (19.4)	19 (61.3)	6 (19.4)	
CRBSI	6 (35.3)	9 (52.9)	2 (11.8)	
Genitourinary	1 (25.0)	0 (0)	3 (75.0)	
Unknown	7 (25.9)	13 (48.1)	7 (25.9)	

RESULTS

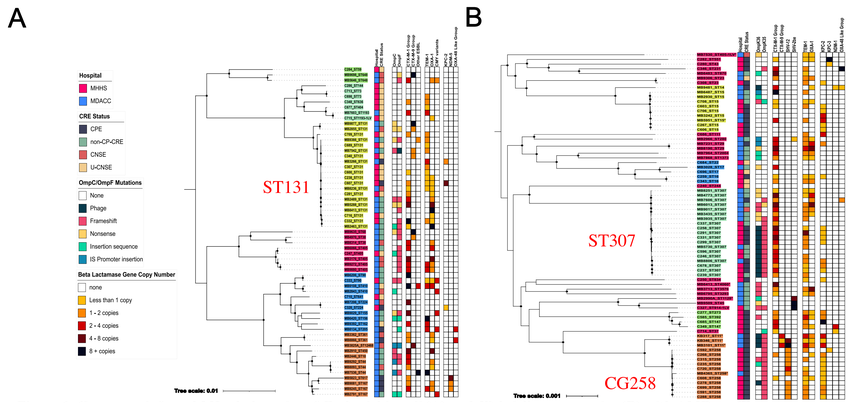

Figure 1. Core gene inferred ML phylogenies of *Escherichia coli* (A) and *Klebsiella pneumoniae* (B) with AMR profile. Background tip label corresponds to hierarchical cluster group with the suffix indicating sequence type. Legend indicates (1) hospital; (2) CRE status; (3) Outer membrane promoter specific mutations; (4) estimated copy number of beta lactamase encoding genes

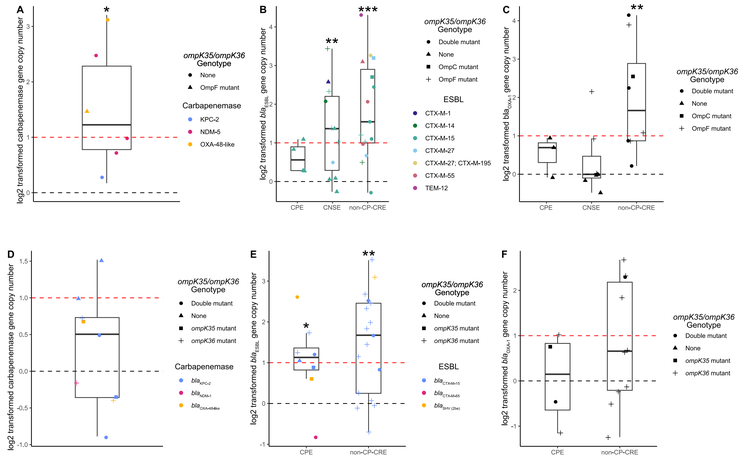

Figure 2. Carbapenemase, ESBL, and bla_{OXA-1} gene encoding copy number estimates with outer membrane porin (omp) mutation categories for *Escherichia coli* (A-C) and *Klebsiella pneumoniae* (D-F) isolates. Dotted horizontal black and red line corresponds to 1 and 2 gene copies respectively. CPE = carbapenemase producing *Enterobacterales*; CNSE = carbapenem-non-susceptible *Enterobacterales*; non-CP-CRE = non-carbapenemase-producing carbapenem resistant *Enterobacterales;* Wilcoxon signed-rank test p-value: *<0.05, **<0.01,***<0.001

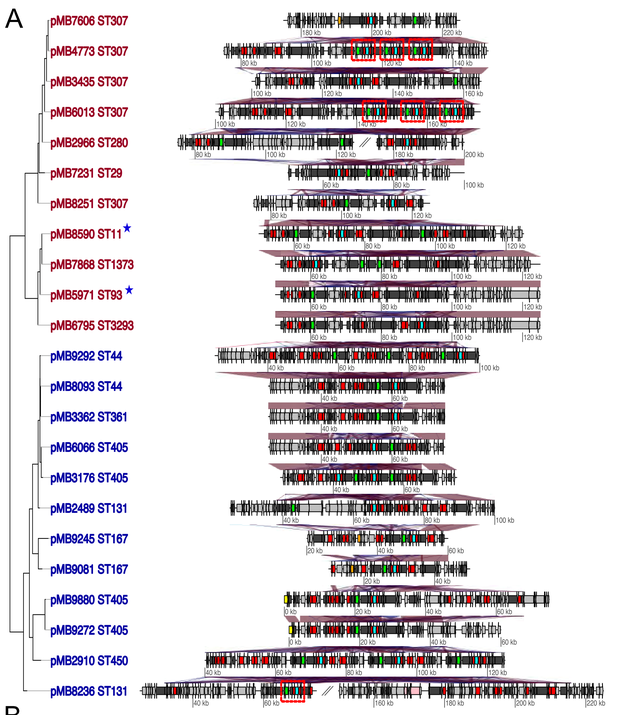

Figure 3. (A) Multi-replicon F-type Vectors of $bla_{CTX-M-15}$ and bla_{OXA-1} Co-Carriage Regions. Neighbor joining tree of completely resolved F-type plasmids with tip-labels indicating whether carried by *Klebsiella spp.* (red) and *Escherichia coli* (blue) with sequence type indicated as suffix. Blue stars indicate non-*Klebsiella pneumoniae* isolates (pMB5890 = *K. michiganesis* and pMB5971 = *K. aerogenes*). $bla_{CTX-M-15}$ (green), bla_{OXA-1} (blue) carbapenemase (orange), virulence factor (pink), AMR encoding gene (red), mobile genetic element (dark grey), other (light grey) are color coded appropriately. Blastn identities > 90% in direct and inverse orientations are shown in red and blue respectively. MB8590 is only unconfirmed CRE isolate included. **(B)** IS*26* composite transposon found in *E. coli* ST131 and *K. pneumoniae* ST307 isolates highlighted in dotted red squares in figure (A).

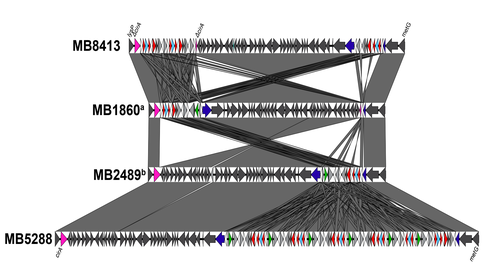

Figure 4. Chromosomal amplifications of $bla_{CTX-M-15}$ and bla_{OXA-1} on *E. coli* ST131 subclade. Genomic context of similar IS*26* translocatable units with genomes indicated a/b superscripts from previous article found in Shropshire *et al*. 2020 JAC. $bla_{CTX-M-15}$ (green), bla_{OXA-1} (blue), IS*26* transposase (white) are labelled accordingly.

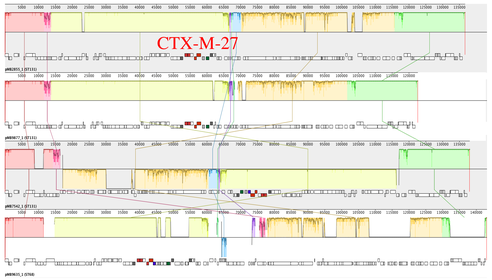

Figure 5. Comparison of $bla_{CTX-M-27}$ F-type plasmid vectors. Genomic context of similar IS*26* translocatable units carrying $bla_{CTX-M-27}$ (green) co-localizing with other AMR encoding genes (red) with colinear syntenic blocks (CSBs) color coded for each respective F-type plasmid. (CSBs) below plane (e.g. pMB7542) indicates inversion of region.

CONCLUSIONS

- There are statistically significant ESBL amplifications with concomitant outer membrane porin mutation genetic backgrounds in both non-CP-CR *E. coli* and *K. pneumoniae* BSI isolates.
- IS*26* composite transposons on F-type plasmids are primary drivers of ESBL amplifications that are associated with carbapenem resistance development.
- Future analyses will focus on how highly successful multi-drug resistant lineages such as *E. coli* ST131 and *K. pneumoniae* ST307 can initially adapt to carbapenem exposures using these non-carbapenemase molecular mechanisms.

Skf System, a Promising New Bacteriocin System of Staphylococcus aureus

Portela R[1,2], Silva MA[1,2], Gonçalves BV[1,2], Dias JD[1,2], Rosato A[3], Rita G. Sobral[1,2]
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2. UCIBIO, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal
3. Riverside University Health System, University of California, Riverside, CA
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Background. We recently discovered by transcriptomic studies that the *murF* mutant F9, constructed in MRSA COL background strain, showed the striking over-expression of two small genes, unannotated in databases and encoding for two low molecular weight peptides (<10 kDa) with high isoelectric points. Moreover, besides *murF* disruption, F9 harbored a loss-of-function mutation in a transcription termination factor that limited the amplification of the plasmid, inserted in several tandem copies. Data in the literature showed that the expression of the two small genes is affected by the transcription factor and belongs to the same regulon of competence-related genes, suggesting that it may be activated by eDNA integration into *S. aureus* genome. A third gene, located downstream and also overexpressed, encodes a putative exporter; the 3 genes co-locate in most sequenced Staphylococci and are predicted to be functionally related.

Hypothesis. Our hypothesis is that these two small peptides may be a two-peptide bacteriocin system, that we named Skf; the small peptides were designated SkfA and Skfb and the putative transporter, SkfC. The peptides were searched in bacteriocin databases and, to our current knowledge, have not yet been described.

Methods. To determine a possible interaction with lipidic membranes, recombinant versions of the peptides were produced and analysed by NMR. Also *skfA/B* knock-out and over-expression mutants were constructed and characterized regarding growth and macrophage internalization.

Results. Secondary structure analysis predicted the presence of an amphipathic helix in each peptide, a pattern often associated with adsorption at polar-apolar interfaces. The 1H-15N HSQC spectra showed poor signal dispersion typical of intrinsic disordered proteins and changes in the chemical shifts were observed in the presence of a *S. aureus* lipid emulsion. A similar effect was observed with anionic membrane mimetics but not with non-charged detergents indicating that the peptides can associate to membranes, probably through an inducible amphipathic a-helix.

Knock-out mutants for each gene showed no altered phenotypes of growth or resistance. However, conditional overexpression mutants for the two small genes showed a strong detrimental impact on *S. aureus* growth. This effect increased with the inducer concentration and with the co-expression of the two genes simultaneously, showing a synergistic activity. Addition of Mg2+ amounts described to permeabilize *S. aureus* membrane, partially rescued the growth inhibition. All together these results could suggest that sfkA/B have inhibitory effects against the cell membrane of *S.aureus*. Furthermore, the overexpression of the peptides promoted a 5-fold increase in internalization in THP1 macrophages

Conclusions. New strategies are urgently needed to deal with the constant rise of multidrug resistant strains including MRSA. Bacteriocins emerge as interesting approaches, as they act by membrane disruption, with demonstrated activity against multiresistant pathogens and with low propensity of resistance selection. The Skf system is a promising candidate.

Acknowledgements. The work was financed by national funds (FCT) by project UIDP/04378/2020 and UIDB/04378/2020 (UCIBIO) and project LA/P/0140/2020 (i4HB) and grant PTDC/BIA-MIC/31645/2017. Norma transitória DL 57/2016 Contract to JSD. NMR spectrometers, of PTNMR, are partially supported by Infrastructure Project No22161 (FEDER by COMPETE 2020, POCI and PORL and FCT by PIDDAC).

Skf System, a Promising New Bacteriocin System of *Staphylococcus aureus*

Portela R.[1,2], Silva M.[1,2], Gonçalves B.V. [1,2], Dias J.S.[1,2], Rosato A.[3], Sobral R.[1,2]

[1]Laboratory of Molecular Microbiology of Bacterial Pathogens, UCIBIO - Applied Biomolecular Sciences Unit, School of Science and Technology, NOVA University Lisbon, Caparica, Portugal
[2]Associate Laboratory i4HB – Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, Caparica, Portugal
[3] Riverside University Health System, University of California, Riverside, CA

Background

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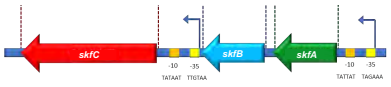
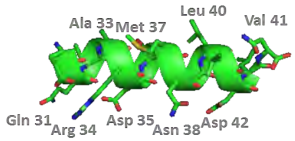
Hypothesis. Our hypothesis is that these two small peptides may be a two-peptide bacteriocin system, that we named Skf; the small peptides were designated SkfA and Skfb and the putative transporter, SkfC. The peptides were searched in bacteriocin databases and, to our current knowledge, have not yet been described.



32.4 kDa 8.2 kDa 7.8 kDa

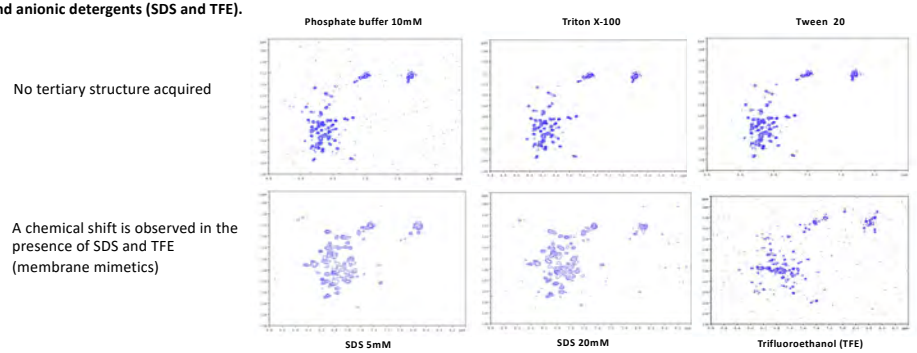
Structural analysis by NMR

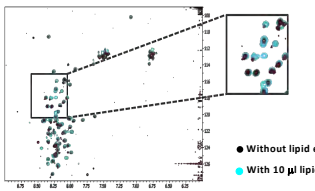


Secondary structure analysis predicted the presence of an amphipathic helix in each peptide, a pattern often associated with adsorption at polar-apolar interfaces.

Model prediction (SWISS MODEL) of an α-helix of SkfB protein of amphiphatic nature with hydrophilic (lower face) and hydrophobic residues (upper part).

^{1}H-^{15}N-HSQC NMR spectrum of SkfB protein in the presence of non-anionic detergents (phosphate buffer, Triton X-100 and Tween 20) and anionic detergents (SDS and TFE).





^{1}H-^{15}N-HSQC NMR spectrum of SkfB protein in the presence of a lipid emulsion.

Changes in the chemical shifts were observed for SkfB peptide in the presence of a *S. aureus* lipid emulsion.

Evidence that SkfB can associate to membranes

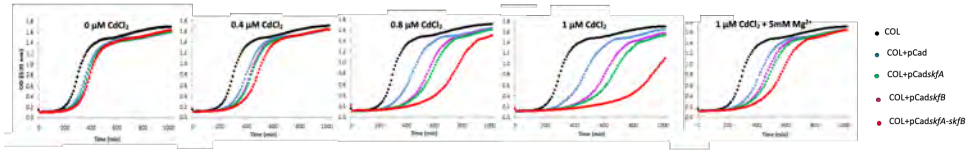
References

1. Sobral R.G., A.M. Ludovice, S. Gardete, K. Tabei, H. de Lencastre and A. Tomasz. 2003. *Microb Drug Resist*. 9: 231-241.

Impact of Skf system on *S. aureus* growth

A knock-out mutant for *skfA* and *skfB* genes and a conditional mutant for *skfC* were constructed and the down-regulation of these genes showed no impact on the growth curve of *S. aureus*.

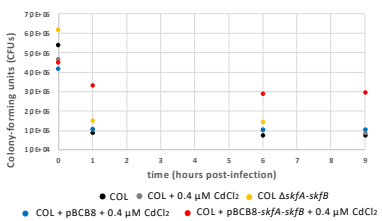
However, over-expression of *skfA* and *skfB* genes, using a $CdCl_2$ inducible promoter, resulted in a decrease in the growth rate that was more evident when both genes were over-expressed. Moreover, addition of Mg^{2+} to the medium seemed to rescue this effect, suggesting that the SkfA/B proteins may be exported.



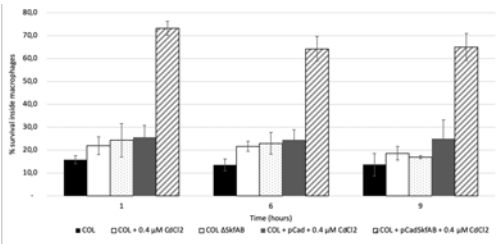
Growth curves of *skfA*, *skfB* and *skfA/B* overexpression mutants in the presence of different concentrations of the inducer $CdCl_2$.

Impact of SkfA/B in the capacity of internalization of *S. aureus* by human macrophages THP-1

The internalization capacity of COL by human macrophages THP-1 was evaluated through an infection assay. The macrophages were infected with the knock-out mutant COLΔ*skfA-skfB* and with the overexpression mutant COL+pBCB8-*skfA-skfB* (multiplicity of infection = 5) grown in the presence of 0.4 µM of $CdCl_2$ inducer. The number of colony-forming units and the percentage of internalized cells in the macrophages was calculated at different timepoints.



Number of CFUs for COL, COL+0.4 µM $CdCl_2$, COLΔ*skfA-skfB*, COL+pBCB8 grown with 0.4 µM $CdCl_2$ and COL+pBCB8-*skfA-skfB* grown with 0.4 µM $CdCl_2$ at different timepoints after infection.



Percentage of internalization inside macrophages THP-1 for COL, COL grown with 0.4 µM $CdCl_2$, COLΔ*skfA-skfB*, COL+pBCB8 grown with 0.4 µM $CdCl_2$ and COL+pBCB8-*skfA-skfB* grown with 0.4 µM $CdCl_2$ at different timepoints after infection.

No changes in internalization or survival in macrophages were observed for the *skfA/B* knock-out mutant. However, a 4.6x increase in the internalization capacity of macrophages for COL+pBCB8-*skfA-skfB* induced with 0.4 µM $CdCl_2$, was observed compared to the parental strain, suggesting a role for the Skf system in host interaction.

Conclusions

- **In silico and NMR data suggest that SkfA and SkfB peptides have the capacity to interact with biological membranes.**
- **SkfA and SkfB peptides seem to be exported from *S. aureus* cell, probably through the action of SkfC transmembrane protein.**
- **The overexpression, but not the absence of *skfA* and *skfB* genes is detrimental for *S. aureus* growth in liquid medium.**
- **The overexpression, but not the absence of *skfA* and *skfB* genes favors the internalization and survival of *S. aureus* in human macrophages THP-1.**

Acknowledgments

This work was financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB. This work was also supported by FCT through grant PTDC/BIA-MIC/31645/2017 (awarded to R.G.S.).

Norma transitória DL 57/2016 Program Contract to JSD. The NMR spectrometers are part of the National NMR Network (PT NMR) and are partially supported by Infrastructure Project No 22161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC).

Developing Methyl Halide Transferase-Based Gas Reporter as a Novel Growth Quantification Approach for Bacteria in Emulsion Droplets

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Background

Applications of microfluidic techniques in experimental evolution is a rapidly emerging field. Water-in-oil emulsion microdroplets can serve as controllable micro-vessels for bacterial evolution studies. To evaluate bacterium growth without disrupting the structure of microdroplets, various color, fluorescence, and luminescence-based reporters have been engineered. Fluorescence-based reporters, however, suffer from important limitations when used within emulsions. First, optics-based signals can have high background noise from the culture media and oil-aqueous interface. Sensitivity can be further reduced during early incubation when the population density of cells is low. Second, optics-based detection is not amenable to filamentous bacteria, biofilm-producing bacteria and opaque media. In this study, we propose that volatile methyl halide gases (CH_3X) produced by reporter cells harboring a methyl halide transferase (MHT) expression cassette can serve as an alternative non-optics detection approach, particularly suitable for bacteria cultured in emulsion droplets. MHT can synthesize methyl halide gases with methyl donor S-adenosyl methionine and halide ions (Br^-), and the methyl halides production can be quantified by gas chromatography-mass spectrometry (GC-MS). In this study, an MHT-labeled *Streptomyces venezuelae* reporter strain was constructed and characterized as a proof-of-concept.

Goal

We aim to demonstrate the feasibility of using volatile methyl halide production as a novel approach to quantitatively characterizing bacterium growth inside water-in-oil emulsion droplets.

Methods

This study uses a polydimethylsiloxane (PDMS)-based droplet generator for encapsulation of bacterium cells into mono-dispersed water-in-oil emulsion droplets. Methyl halide production was detected by GC-MS. MHT expression cassette was knocked into *S. venezuelae* genome via ϕC-31-mediated integration.

Results

An MHT expression vector was constructed and introduced into *S. venezuelae* via *Escherichia coli*-mediated conjugation. The MHT cassette was then integrated into *attB* sites of the *S. venezuelae* genome through a ϕC-31-mediated integration mechanism. Methyl bromide (MeBr) production was detected using 0.1 M sodium bromide (NaBr) as substrate by GC-MS. Production of MeBr from reporter cells cultured in both suspension and ϕ150 μm emulsion microdroplets was monitored for 24 h. Within 8 h, a linear correlation between MeBr production and incubation time was observed for reporters in suspension whereas the correlation was exponential for reporters in emulsion microdroplets. To estimate cell density from MeBr production, standard calibration curves were established. A linear correlation between cell density and MeBr production was observed for suspension conditions whereas the correlation was semi-log for emulsion microdroplet conditions. The sensitivity of the MeBr production approach was quantified. The signal to noise ratio of the gas reporter was found to be 2 to 3 orders of magnitude higher than the optics-based reporters within 24 h of incubation. Further, it was demonstrated that gas reporters could be used to reliably detect a cell density as low as 3 million/mL.

Conclusion

We have demonstrated the feasibility of using volatile methyl halide production as a non-optics reporter for bacterium growth inside water-in-oil emulsion droplets.

Acknowledgement

Funding source: National Institutes of Health R01 A1080714 to Prof. Yousif Shamoo

Developing Methyl Halide Transferase-Based Gas Reporter as a Novel Growth Quantification Approach for Bacteria in Emulsion Droplets

Xinhao Song and Yousif Shamoo
Department of Biosciences, Rice University, Houston, TX, USA

Background

Applications of microfluidic techniques in experimental evolution is an emerging field of study and water-in-oil emulsion droplets could serve as micro-vessels for bacteria incubation and evolution. To evaluate bacterium growth without disrupting the structure of droplets, various color, fluorescence, and luminescence-based reporters have been engineered. Optics-based reporters, however, suffer from some limitations. First, optics-based signals are interfered by the background noise from culture media and oil-aqueous interface. The sensitivity would be further reduced during early incubation stages when the population density of reporters is low. Second, optics-based detection is not applicable to filamentous bacteria, biofilm-producing bacteria and bacteria incubated in opaque media. In this study, we propose that volatile methyl halide gases (CH_3X) produced by reporter cells harboring a methyl halide transferase (MHT) expression cassette can serve as an alternative non-optics detection approach, particularly suitable for bacteria cultured in emulsion droplets. MHT can synthesize methyl halide gases with methyl donor S-adenosyl methionine and halide ions (Br^- in this study), and the methyl halides production can be quantified by gas chromatography-mass spectrometry (GC-MS). In this study, an MHT-labeled *Streptomyces venezuelae* reporter strain was constructed and characterized as a proof of concept.

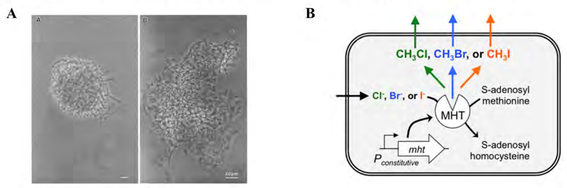

Figure 1 (A) Typical streptomycete morphology in liquid culture as represented by *S. lividans* 1326. (Hobbs et al., 1989) **(B)** Methyl halide transferase (MHT) synthesizes methyl halide gases (CH_3Cl, CH_3Br and CH_3I) with methyl donor S-adenosyl methionine and halide ions (Cl^-, Br^- and I^-, respectively). (Cheng, 2016)

Goal

The aim of this project is to demonstrate the feasibility of using volatile methyl halide production as a novel approach to quantitatively characterizing bacterium growth inside water-in-oil emulsion droplets.

Method

PDMS-based microfluidic devices are manufactured from silicon wafer molds

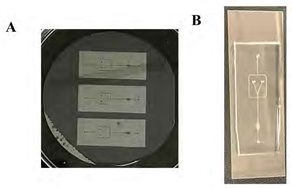

Figure 2（**A**）Molds of microfluidic device are made from silicon wafer covered with photoresist SU-8 2075. Features are curved by UV-photolithography in a cleanroom facility at Rice University (**B**) Polydimethylsiloxane (PDMS)-based microfluidic devices are manufactured from silicon wafer molds, and then attached to a piece of glass cover glass using plasma activation.

Results

Streptomyces venezuelae gas reporter was engineered by φC-31 integration mechanism and encapsulated into emulsion droplets

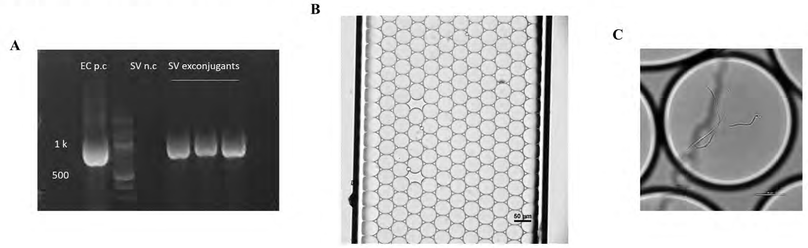

Figure 3 (A) *Streptomyces venezuelae* gas reporter strain was constructed by knocking-in the MHT-expressing cassette into the *attB* sites via φC31 integration mechanism. Colony PCR confirmed the presence of MHT-expressing cassette in the genome of *S. venezuelae* exconjugants. (**B**) *S. venezuelae* gas reporters were encapsulated into mono-dispersed water-in-oil emulsion microdroplets of ~50 μm diameter. **(C)** An enlarged image showing one emulsion microdroplet containing mycelium fragments of MHT-labeled *S. venezuelae*.

Methyl bromide (MeBr) production was detected using GC-MS from _S. venezuelae_ gas reporter incubated in both liquid suspension and emulsion microdroplets

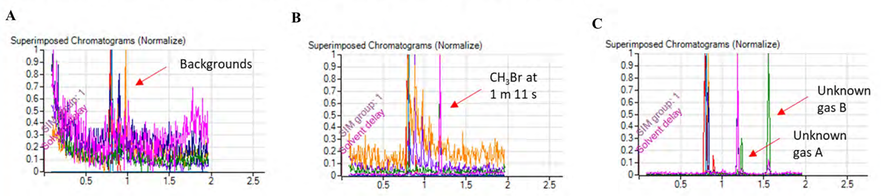

Figure 4 Gas chromatography-mass spectrometry detection of MeBr for (**A**) wildtype *S. venezuelae*, (**B**) *S. venezuelae* gas reporter incubated in liquid suspension and (C) *S. venezuelae* gas reporter incubated in emulsion microdroplets. MeBr production was detected at 1 min 11 s post injection only for *S. venezuelae* gas reporter incubated in both conditions.

Methyl bromide (MeBr) accumulation was linear in liquid suspension whereas exponential in emulsion microdroplets

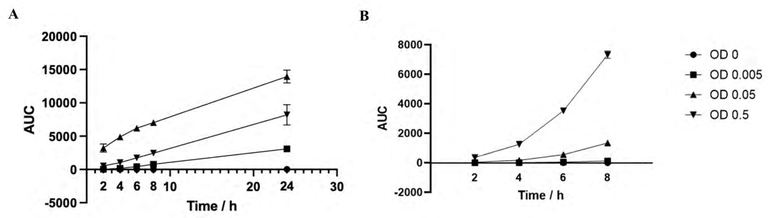

Figure 5 (**A**) MeBr accumulation from *S. venezuelae* gas reporter incubated in liquid suspension. The accumulation curves of three initial cell densities were all linear over time. (**B**) MeBr accumulation from *S. venezuelae* gas reporter incubated in emulsion microdroplets. The accumulation curves of three initial cell densities were all exponential over time.

Methyl bromide (MeBr) production assay yields better signal to noise (S/N) ratio than optics-based approaches

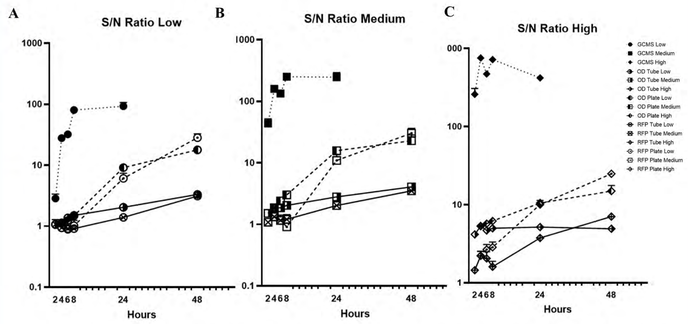

Figure 6 MeBr production assay yielded better S/N ratio compared to optical density assay and fluorescence assay. For the two optics-based assays, an *S. venezuelae* RFP reporter strain constructed using the same protocol as the gas reporter was incubated in either sealed 2 mL tubes or a 96-well microplate. Better S/N ratios were observed in all three initial population densities tested.

Methyl bromide (MeBr) production can be used as a quantitative assay for both liquid suspension and emulsion microdroplet conditions

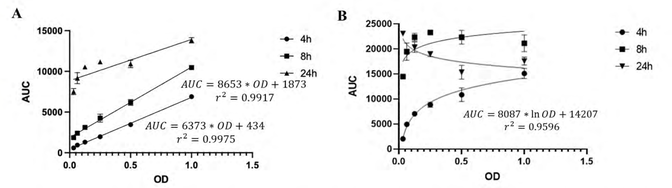

Figure 7 (A) Standard curve for MeBr production (AUC determined by GC-MS) to initial population density (OD). Linear correlations were observed ($r^2 > 0.99$) after 4 or 8 hours of gas accumulation. (**B**) Standard curve for MeBr production (AUC determined by GC-MS) to initial population density (OD). A semi-log correlation was observed ($r^2 > 0.95$) after 4 hours of gas accumulation.

Conclusion

We have demonstrated the feasibility of using volatile methyl halide production as a non-optics reporter for bacterium growth inside water-in-oil emulsion droplets.

Acknowledgement

Funding: National Institutes of Health R01 A1080714 to Prof. Yousif Shamoo

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Molecular Characteristics of Emergent Extended-Spectrum Beta-Lactamase Escherichia Coli Infections at MD Anderson Cancer Center

Strope B[1], Shropshire WC[3], Kalia A[1], Flores A[2], Gohel M[3], Aitken S[2,4], McDaneld P[3], Sahasrabhojane P[3], Shelburne SA[2,3,5]

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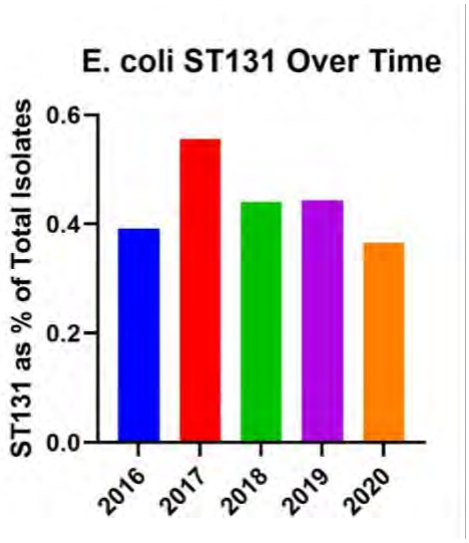
Background: *Escherichia coli*, a Gram-negative bacterium, is the leading cause of invasive bacterial infections at MD Anderson Cancer Center (MDACC). These infections pose various challenges with respect to their management, especially for antimicrobial resistant (AMR) isolates. Over the past five years there has been an increase in the rates of extended-spectrum β-lactamase *E. coli* (ESBL-EC) infections at MDACC, which mirrors a similar increase noted nationwide. The primary objective of this study is to use a genomic level analysis to determine the genetic relationship amongst ESBL-EC bacteremia isolates at MDACC.

Hypothesis/Goals: An increased prevalence of *E. coli* sequence type 131 (ST131) harboring $bla_{\text{CTX-M-15}}$ accounts for the increase in EBSL-EC bacteremia observed from 2016 to 2020 at MDACC.

Methods: We defined ESBL-E positive isolates as having a ceftriaxone (CRO) MIC of ≥2 μg/mL based on CLSI M100 Guidelines (2018). We abstracted ceftriaxone resistant (CRO-R) *E. coli* bacteremia isolates from unique patients collected from January 2016 – December 2020 using the Epic Systems electronic medical record software and cross referenced those with isolates present in our Microbe Bank. We identified 352 isolates that met thesei criteria. Isolates were whole genome sequenced via the Illumina Sequencing Nova6000 platform. Bioinformatics analysis included species identification, multi-locus sequence typing (MLST), antimicrobial resistance (AMR) gene detection, plasmid sequence identification, phylogenetic analysis, and AMR gene copy number amplification.

Results: We have sequenced 136 CRO-R *E. coli* isolates to date. Our sequence type distribution is as follows: ST131 (41%), ST38 (6.6%), ST405 (6.6%), ST648 (6.7%), and other (39%). CTX-M enzymes were the most common cause of the ESBL phenotype, with 85% of strains harboring a variant of this enzyme. Our most common β-lactamase encoding genes were $bla_{\text{CTX-M-15}}$ (55%), $bla_{\text{OXA-1}}$ (33%), $bla_{\text{TEM-1}}$ (24%), $bla_{\text{CTX-M-55}}$ (16%), $bla_{\text{CTX-M-27}}$ (13%), and $bla_{\text{CTX-M-14}}$ (9%). Additionally, a majority (63%) of ESBL strains harbored more than one β-lactamase encoding gene. Nearly all ST131 isolates harbored a CTX-M enzyme (98%). ST131 prevalence remained stable across the five years (Figure 1) with sporadic introductions of other less prevalent MLST groups.



Conclusions: Our preliminary findings suggest stable prevalence of ST131 with no clear clonal emergence. Further sequencing of the cohort will assist with clarifying the mechanisms driving the increasing ESBL infections observed in our patients, which may be reflective of nationwide trends. Currently, our data suggest that the increase in ESBL infections at MD Anderson was due to diverse sequence types harboring various ESBL enzymes.

Molecular Characteristics of Emergent Extended-Spectrum Beta-Lactamase *Escherichia Coli* Infections at MD Anderson Cancer Center

Strope B[1], Shropshire WC[3], Kalia A[1], Flores A[2], Gohel M[3], Aitken S[2,4], McDaneld P[3], Sahasrabhojane P[3], Shelburne SA[2,3,5]

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INTRODUCTION

- *Escherichia coli* is the leading cause of invasive bacterial infections at MD Anderson Cancer Center (MDACC)
- Over the past five years there has been an increase in the rates of extended-spectrum β-lactamase *E. coli* (ESBL-EC) infections at MDACC
- This increase in ESBL-EC prevalence is a growing trend within US hospitals
- The primary objective is to use a genomic level analysis to determine the genetic relationship amongst ESBL-EC bacteremia isolates at MDACC

HYPOTHESIS

An increased prevalence of *E. coli* sequence type 131 (ST131) harboring $bla_{CTX\text{-}M\text{-}15}$ accounts for the increase in EBSL-EC bacteremia observed from 2016 to 2020 at MDACC.

METHODS

- We defined ESBL-E positive isolates as having a ceftriaxone (CRO) MIC of ³2 µg/mL based on CLSI M100 Guidelines (2018).
- We identified 352 isolates that met these criteria.
- Isolates were whole genome sequenced via the Illumina Sequencing Nova6000 platform.
- Bioinformatics analysis included:
  - species identification, multi-locus sequence typing (MLST), antimicrobial resistance (AMR) gene detection, plasmid sequence identification, phylogenetic analysis, and AMR gene copy number amplification.

Year	Organism	Isolate # overall	Isolates + for ESBL on testing	ESBL+ %
2016*	e coli	1017	143	14.1%
2017	e coli	1993	319	16.0%
2018	e coli	2025	372	18.4%
2019^	e coli	1615	330	20.4%

Table 1: Increase in ESBL positive *E. coli* prevalence at MDACC from 2016 – 2019. The ESBL+ prevalence increased from 14.1 to 20.4% from 2016 to 2019. This mirrors a similar worldwide trend established by the CDC.

RESULTS

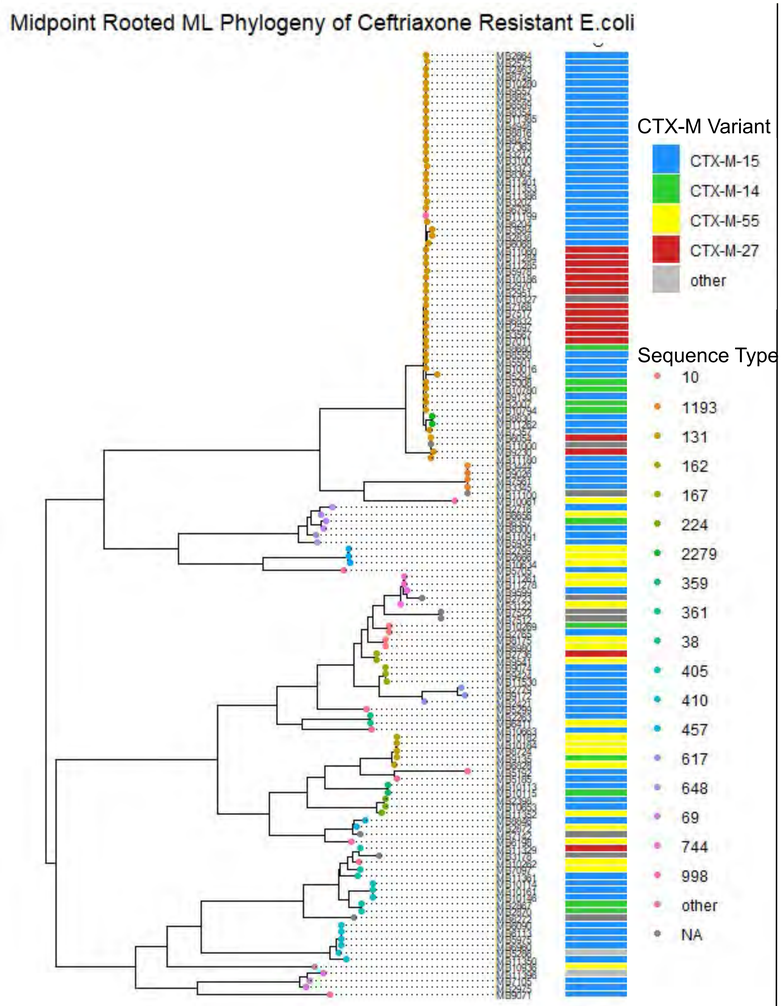

Figure 1: Population structure of ceftriaxone (CRO) resistant *Escherichia coli* (n=136). Tip labels correspond to sequence type with column corresponding to CTX-M variant identified. Large association of ST131 with CTX-M-15 and CTX-M-27 enzymes. There is a more diverse association of CTX-M-Enzymes outside of ST131 clade.

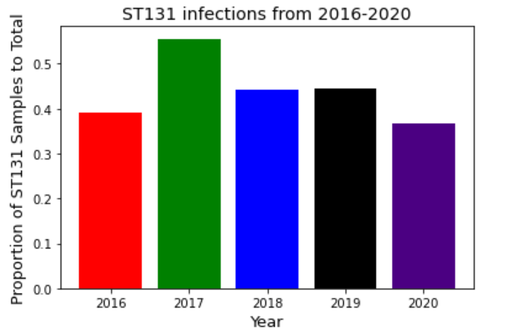

Figure 2: Temporal trend of ST131 prevalence. No association between increase/decrease of ST131 over five-year period. No significant difference in the counts of ST131 samples each year (Kruskal-Wallis chi-squared = 4, df = 4, p-value = 0.406)

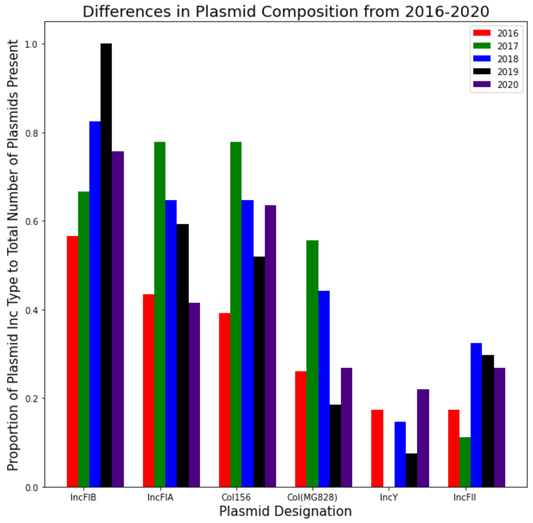

Figure 3: Distribution of plasmid replicon types over time. Stable presence of F-type plasmids. No strong association between plasmid type and AMR gene composition by year. Suggests an introduction of various sequence types harboring diverse resistance mechanisms in MDACC population

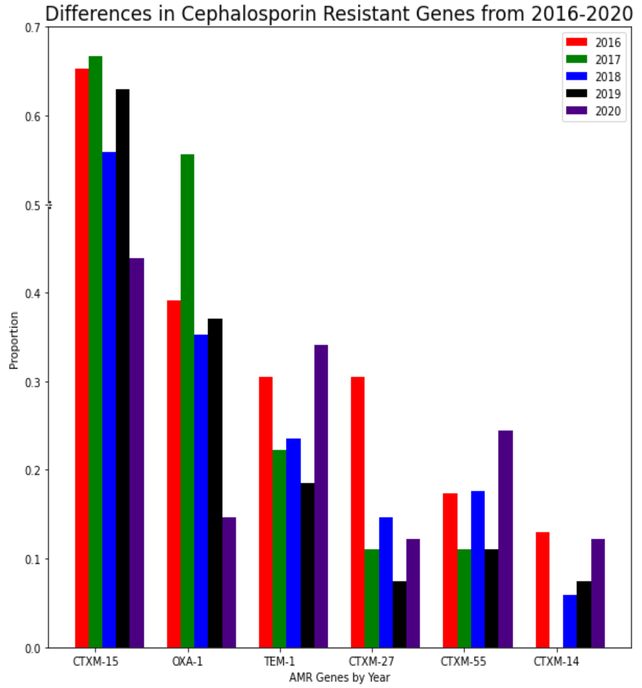

Figure 4: Beta-lactamase Gene Distribution over five—year period. No significant correlation between any CTX-M enzyme and plasmid type (p>0.05, Pearson's correlation)

CONCLUSION

- Stable prevalence of ST131 with no clear clonal emergence of other high-risk clone
- Preliminary data suggests that the increase in ESBLs was due to diverse sequence types harboring various ESBL enzymes
- Further sequencing of the cohort will assist with clarifying the mechanisms driving the increasing ESBL infections observed in our patients

ACKNOWLEDGMENTS

- This project was supported by MD Anderson CCSG Cancer Research Career Enhancement component (P30 CA016672).
- BS was supported by MD Anderson Cancer Center Emergency Funding under CARES Act

LiaX is Essential for Cell Envelope Adaptation via the LiaFSR System in Enterococcus faecium

Tran TT[1,2], Panesso D[1,2], Zhao A[3], Axell-House DB[1,2], Arias CA[1,2]

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Background: Daptomycin (DAP) is a bactericidal lipopeptide antibiotic that is often used for treatment of severe enterococcal infections. Our previous studies have identified LiaX as a surface-exposed protein that is an important mediator of cell envelope homeostasis in *E. faecalis* upon exposure to DAP and other antimicrobial peptides via activation of the LiaFSR three-component regulatory system. LiaX encodes for a soluble protein with two distinct domains, an N-terminal which is predicted to harbor α-helices and a C-terminus which is β-pleated sheets. Its role in *E. faecium*, a species of clinical relevance, remains unknown. In this work, we aim to elucidate the function of LiaX of *E. faecium*.

Methods: We used the commensal strain of *E. faecium* TX1330RF (DAP minimum inhibitory concentration [MIC] 3 μg/ml), whose genome is sequenced, and its Δ*liaR* derivative, TX1330RFΔ*liaR* (DAP MIC 0.125 μg/ml), and targeted the *liaX* gene for mutagenesis. Using the p-chloro-phenylalanine (p-Chl-Phe) sensitivity counterselection system (PheS*), we aimed to create a truncation or in-frame deletion of *liaX*. All mutants were confirmed by PFGE and sequencing of the entire *liaX* gene. Mutants were characterized by determination of DAP susceptibility by E-test and visualization of anionic phospholipid domains by 10-N-*nonyl*-acridine orange (NAO).

Results: In the presence of *liaR*, we were unable to create truncation or in-frame deletion of *liaX* after many attempts. In *E. faecium* TX1330RFΔ*liaR*, we created an in-frame deletion of *liaX*, TX1330RFΔ*liaRliaX*. DAP MIC of TX1330RFΔ*liaRliaX* remained at 0.125 μg/ml. NAO staining of all strains of TX1330RF, TX1330RFΔ*liaR*, and its Δ*liaX* mutant showed localization of anionic phospholipid domains at septa of the cells. Interestingly, multiple attempts to complement *liaR* in TX1330RFΔ*liaRliaX* were also futile.

Conclusion: Our findings suggest that LiaX may be essential in *E. faecium* whose LiaFSR is intact. Efforts to elucidate its function in *E. faecium* are ongoing.

Acknowledgments: National Institute of Allergy and Infectious Diseases (R01 AI148342-02) to Dr. Cesar A. Arias

LiaX is Essential for Cell Envelope Adaptation via the LiaFSR System in *Enterococcus faecium*

Tran TT[1,2], Panesso D[1,2], Zhao A[3], Axell-House DA[1,2], Arias CA[1,2]

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ABSTRACT

Background: Daptomycin (DAP) is a bactericidal lipopeptide antibiotic that is often used for treatment of severe enterococcal infections. Our previous studies have identified LiaX as a surface-exposed protein that is an important mediator of cell envelope homeostasis in *E. faecalis* upon exposure to DAP and other antimicrobial peptides via activation of the LiaFSR three-component regulatory system. LiaX encodes for a soluble protein with two distinct domains, an N-terminal which is predicted to harbor α-helices and a C-terminus which is β-pleated sheets. Its role in *E. faecium*, a species of clinical relevance, remains unknown. In this work, we aim to elucidate the function of LiaX of *E. faecium*.
Methods: We used the commensal strain of *E. faecium* TX1330RF (DAP minimum inhibitory concentration [MIC] 3 µg/ml), whose genome is sequenced, and its Δ*liaR* derivative, TX1330RΔ*liaR* (DAP MIC 0.125 µg/ml), and targeted the *liaX* gene for mutagenesis. Using the p-chloro-phenylalanine (p-Chl-Phe) sensitivity counterselection system (PheS*), we aimed to create a truncation or in-frame deletion of *liaX*. All mutants were confirmed by PFGE and sequencing of the entire *liaX* gene. Mutants were characterized by determination of DAP susceptibility by E-test and visualization of anionic phospholipid domains by 10-N-*nonyl*-acridine orange (NAO).
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Conclusion: Our findings suggest that LiaX may be essential in *E. faecium* whose LiaFSR is intact. Efforts to elucidate its function in *E. faecium* are ongoing.

INTRODUCTION

- Enterococci are among the leading causes of hospital-associated infections including urinary tract, bloodstream, intra-abdominal and surgical site infections [1,2].
- Daptomycin (DAP) is a cyclic lipopeptide antibiotic that has *in vitro* bactericidal activity against enterococci, including vancomycin-resistant strains. However, evolving resistance to daptomycin during therapy has been reported in infections caused by *E. faecalis* and *E. faecium* [3-6].
- The LiaFSR stress response system has been shown to orchestrate DAP and antimicrobial peptide resistance in *E. faecalis* by modulating cell membrane phospholipid content and localization.
- LiaX, the effector of the LiaFSR system in *E. faecalis*, senses antimicrobial molecules and regulates changes in cell membrane phospholipid architecture.
- The function of LiaX in *E. faecium*, a species of clinical significance, remains to be elucidated.

AIM

To gain insights into the functional role of *liaX* in *Enterococcus faecium*

METHODS

Bacterial strains –*Enterococcus faecium* TX1330RF and its *liaR (*TX1330RFΔ*liaR)* derivative were used to generate derivatives (**Table 1**).

Mutagenesis Strategy – In-frame and partial deletions of *liaX* were attempted using the *p*-chlorophenylalanine (p-Chl_Phe) sensitivity counterselection system (PheS*) to obtain the mutants. Briefly, ~500 bp regions upstream and downstream of *liaX* were amplified by crossover PCR using DNA from the corresponding strain as the target. Each fragment is cloned into pHOU1 using EcoRI and BamHI. The recombinant plasmids were electroporated into *E. faecalis* CK111 and delivered into rifampicin-resistant derivatives of the target *E. faecium* strain by conjugation. First recombinant integrants were selected on gentamicin (150 µg/ml) and rifampin (100 µg/ml) and subsequently plated in medium containing *p*-chlorophenylalanine. Colonies obtained from the counterselection medium were tested by replica plating in the presence of varying DAP concentrations. Candidate colonies were subjected to pulsed-field gel-electrophoresis to confirm their genetic relatedness with the parent strain. Regions of *liaX* was amplified and subjected to Sanger sequencing to confirm deletion(s) and DAP MICs by Etest.

Fluorescence Microscopy – 10-*N*-nonyl acridine orange (NAO) fluorescent dye was obtained from Molecular Probes. In brief, bacterial cells were grown to early exponential phase in BHI broth. Nisin 50 ng/ml and NAO 1 µM were added and bacterial cells were incubated for 3 h at 37°C with rotary shaking. After staining, 8 µl of cells were immobilized in 1% agarose pads on object slides coated with poly-L-lysine. Cells were viewed with a Keyence BZ-X710 fluorescence microscope using FITC (excitation 495 nm, emission 519 nm).

Figure 1. *liaXYZ* of *E. faecium* TX1330RF

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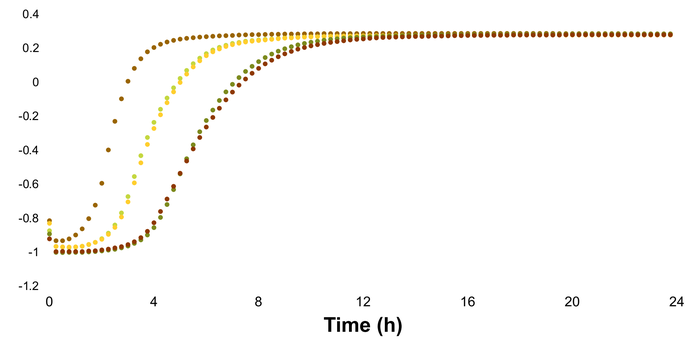
Tran TT, Panesso D, Mishra NN, et al. 2013. mBio **4**:e00281-13
5. **Khan A, Davlieva M, Panesso D, et al** 2019. *Proc Natl Acad Sci U S A*. pii: 201916037

RESULTS

Table 1. *Enterococcus faecium* and derivatives

Strain	DAP MIC (µg/ml)
TX1330RF	1
TX1330RFΔ*liaR*	0.125
TX1330RFΔ*liaR* (pMSP3535::*liaR*)	1
TX1330RFΔ*liaR*Δ*liaX*	0.125
TX1330RFΔ*liaRliaX* (pMSP3535::*liaR*)	0.5

Growth of *E. faecium* Derivatives



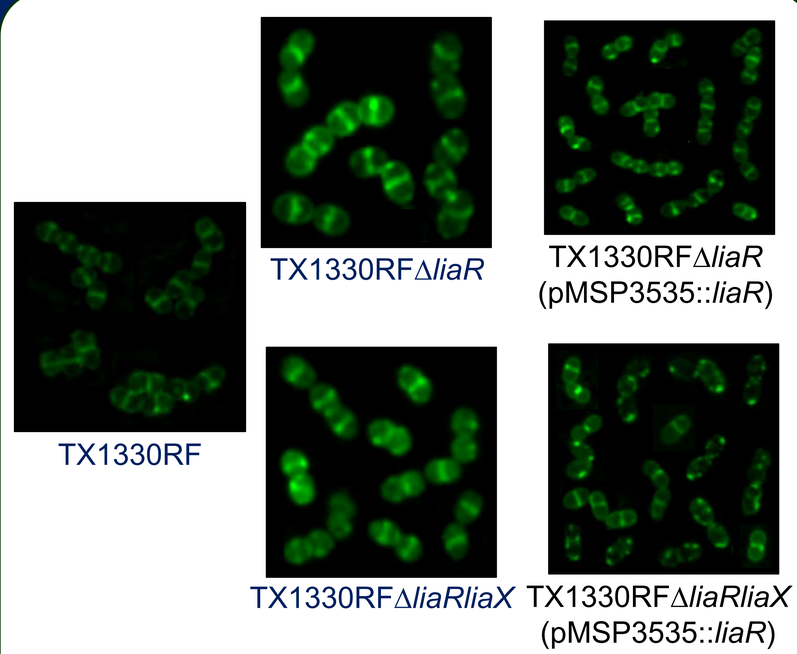


Figure 3. Localization of anionic phospholipids with NAO

CONCLUSION

• Our findings suggest that LiaX may be essential in *E. faecium* whose LiaFSR is intact. Efforts to elucidate its function in *E. faecium* are ongoing.

ACKNOWLEDGMENTS

This work is supported by R01 AI148342-02 (CAA).

Dynamics Of Colonization and Infection By Multidrug-Resistant Pathogens in Immunocompromised and Critically Ill Patients (DYNAMITE): Preliminary Results

Virk MHM[1-2], Rydell KB[1-2], Atterstrom RL[1-2], Jones MN[1-2], deTranaltes AM[1-2], Savidge T[3], Shelburne SA[4], Hanson B[5], Arias CA[1-2]

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Background Antimicrobial resistance is one of the most pressing emerging public health problems across the globe. Of particular interest to this study, vancomycin resistant enterococci (VRE) and extended spectrum beta-lactamase producing Enterobacterales (ESBL-E) are classified by the CDC as serious public health threats, and *Clostridioides dificile* is classified as an urgent threat. The mechanisms of colonization and progression to infection by these organisms are poorly understood.

Goals The goal of the DYNAMITE study is to elucidate the roles of the patient's microbiome and clinical characteristics, as well as antimicrobial resistance in colonization, co-colonization, and infection of VRE, ESBL-E/CRE, and *C. difficile* using longitudinal fecal samples.

Methods To examine the colonization, co-colonization, and infection of VRE, ESBL-E/CRE, and *C. difficile,* potential study subjects are screened from 5 ICUs in Memorial Hermann hospital within 24 hours of their ICU admission. The study subjects who are expected to stay in ICU for 72 hours and do not fall into exclusion criteria (minor, pregnancy, IBD, GI derivation) will be consented to participate in the study. Fecal samples and blood and oral swab samples will be collected twice and once a week respectively, for a maximum of four weeks or until the subject is transferred to a non-ICU floor or home.

Results Our study cohort currently consists of 55 study subjects enrolled from the 5 ICUs of Memorial Hermann hospital. 57% of our study population is male and 58% reported as Caucasian. Most patients (31%) have been enrolled from the Medical ICU followed by the Heart Failure ICU (24%). Our mean study subject enrollment duration is 9.75 days ± 1.13. Study subjects from the cardiac care unit have the highest average study enrollment duration (13.14 days ± 3.20). The average hospital length of stay for our study subjects is 17.55 days ± 1.91. The average number of stool samples per patient is 2.2 ± 0.2 samples. The average number of blood samples per study subject is 1.71 ± 0.13 samples.

Conclusions Our diverse study population and strict standardized sample collection, followed by the use of deep shotgun metagenomics and proteomics, will make clear the roles of the microbiome and antimicrobial resistance in colonization, co-colonization, and infection of VRE, ESBL-E/CRE and *C. difficile.*

Acknowledgments Funded by NIH/NIAID 1PO1AI152999-01. We extend our gratitude towards the directors, physicians, and nursing staff of all the ICUs in Memorial Hermann hospital. We are also grateful to Alejandro De La Hoz Gomez, Dimple Desai, and Christina Abi Kheir for their efforts in writing protocols, screening and enrolling patients, and collecting and accessioning samples.

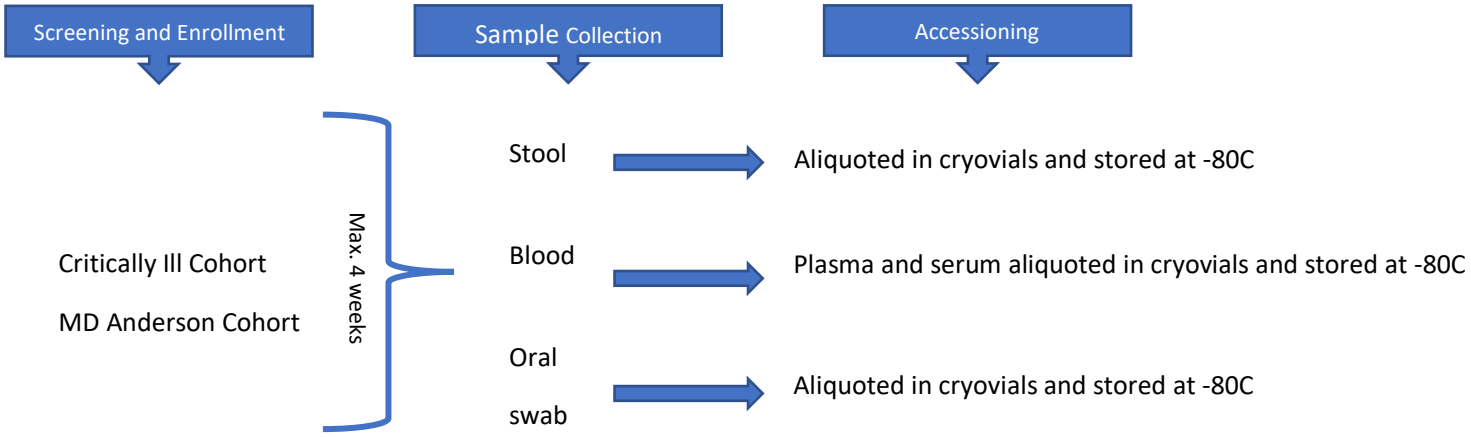

Figure 1 Illustration of screening and enrollment followed by sample collection and accessioning for DYNAMITE study

Dynamics Of Colonization And Infection By Multidrug-Resistant Pathogens In Immunocompromised And Critically Ill Patients (DYNAMITE): Preliminary Results

Virk MHM[1-2], Rydell KB[1-2], Atterstrom RL[1-2], Jones MN[1-2], deTranaltes AM[1-2], Savidge T[3], Shelburne SA[4], Hanson BM[5], Arias CA[1-2]

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3. Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX
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Background

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References

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Methods

Result

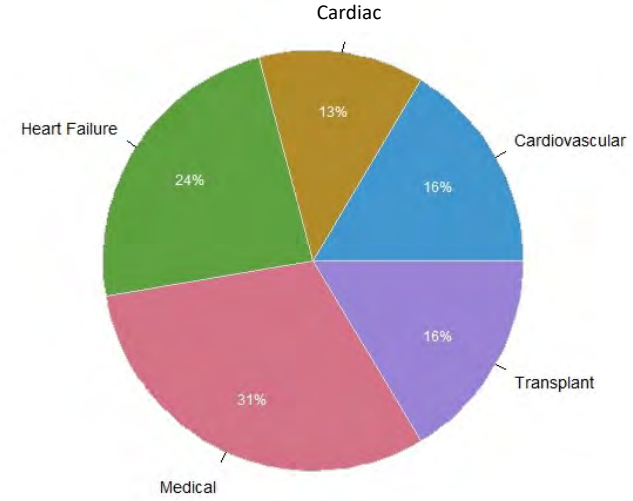

Fig 1. Patient enrollment by ICU

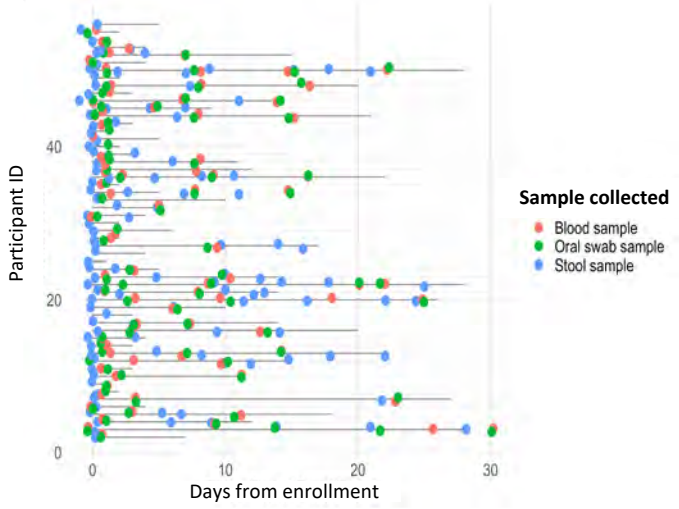

Fig 2. Biological sample collection for each patient

Our study cohort currently consists of 55 study subjects enrolled from the 5 ICUs of Memorial Hermann hospital (Table 1). 57% of our study population is male and 58% reported as Caucasian. Most patients (31%) have been enrolled from the Medical ICU followed by the Heart Failure ICU (24%). (Fig.1). Our mean study subject enrollment duration is 9.75 days ± 1.13. Study subjects from the cardiac care unit have the highest average study enrollment duration (13.14 days ± 3.20). The average hospital length of stay for our study subjects is 17.55 days ± 1.91. The average number of stool samples per patient is 2.2 ± 0.2 samples. The average number of blood samples per study subject is 1.71 ± 0.13 samples. (Fig.2)

Conclusion

Our diverse study population and strict standardized sample collection, followed by the use of deep shotgun metagenomics and proteomics, will make clear the roles of the microbiome and antimicrobial resistance in colonization, co-colonization, and infection of VRE, ESBL-E/CRE and *C. difficile.*

Variables	DYNAMITE Patients
Demographics	
Age (mean ± SD)	60.69 ± 14.23
Gender	
Male (N, %)	30 (54.55%)
Female (N, %)	23 (41.82)
Race	
White (N, %)	32 (58.18%)
African American (N, %)	13 (23.64%)
Origin	
Home (N, %)	35(63.64%)
Transfer (N, %)	14 (25.46%)
Hospital Demographics	
Hospital length of stay (mean ± SD)	19.81 ± 15.89
Study enrollment length of stay (mean ± SD)	11.43 ± 8.89
Reason for Admission	
Cardiovascular (N, %)	16 (29.09%)
Respiratory (N, %)	14 (25.45%)
Gastrointestinal (N, %)	8 (14.55%)
Comorbidities	
Heart Failure (N, %)	25 (45.45%)
Hypertension (N, %)	38 (69.09%)

Table 1. Demographics and hospital stay summary

Customizing Populus Plus Lab Inventory Management System for Large Multi Center Observational trial: DYNAMITE

Virk MHM[1-2], Nick Rutter[3], Jeff Beairsto[3], Atterstrom RL[1-2] Rydell KB[1-2], Jones MN[1-2], Detranaltes AM[1-2], Arias CA[1-2], Hanson B[4]

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Background The Dynamics of Colonization and Infection by Multidrug-Resistant Pathogens in Immunocompromised and Critically Ill Patients (DYNAMITE) study aims to investigate the progression from colonization to infection of drug-resistant pathogens in critically ill patients, and determine the role of the commensal microbiota in this progression. The highly collaborative nature of the study, high volume, longitudinal and hierarchical nature, and diversity of the biospecimens collected make biospecimen accessioning and inventory extremely challenging.

Goals To develop an efficient and customizable LIMS for recording, generating, and integrating data from participating laboratories as well as the primary site of sample collection.

Methods Biospecimens (fecal, blood and oral swab samples) from consented subjects are collected for a maximum of 4 weeks from participant enrollment. All the biospecimens collected are aliquoted into multiple 1.5ml cryovials and stored in several freezer locations which is determined by the biospecimen type and experiment planned. This poses a logistics challenge to accurately accession and aliquot samples into multiple child tubes, as well as to track this parent-child relationship across all experiments and samples. The Populus LIMS is used to store all the data regarding the biospecimens collected. The Populous LIMS also enables storage of biospecimen characteristics such as hemolysis, volume, quality, storage and collection date.

Results To date, we have collected samples from 55 patients at several timepoints throughout their study enrollment. A total of 567 stool aliquots have been processed and stored, along with 640 plasma aliquots, 545 serum aliquots, and 166 oral swab aliquots. These samples have been successfully accessioned, aliquoted, and stored, all while maintaining the parent-child relationship. The custom Populous LIMS serves as an excellent foundation for the DYNAMITE study aims and will enable us to enroll our target of 1000 patients. Sample aliquots are being processed for metagenomics, bacterial culture, and proteomics, with all of the results from these study procedures collected within the LIMS.

Conclusion The Populous LIMS is an extremely efficient cloud-based database that maintains parent-child relationship between the depleted original specimens and their respective aliquots**.** The highly customizable nature of the database provides great opportunity to fine tune the database per the investigator's or research study's demands.

Acknowledgments We are very grateful to the Populus plus team and their web developers for developing LIMS and customizing it per the needs of the DYNAMITE study.

Customizing Populus Plus Lab Inventory Management System for Large Multi-Center Observational trial: DYNAMITE

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Background

The Dynamics of Colonization and Infection by Multidrug-Resistant Pathogens in Immunocompromised and Critically Ill Patients (DYNAMITE) study aims to investigate the progression from colonization to infection of drug-resistant pathogens in critically ill patients, and determine the role of the commensal microbiota in this progression. The highly collaborative nature of the study, large volume of samples, longitudinal and hierarchical nature, and diversity of the biospecimens collected make biospecimen accessioning and inventory extremely challenging.

Goals

To develop an efficient and customizable LIMS for recording, generating, and integrating data from participating laboratories, as well as the primary site of sample collection.

Methods

Biospecimens (fecal, blood and oral swab samples) from consented subjects are collected for a maximum of 4 weeks during participant enrollment. All the biospecimen samples collected are aliquoted into multiple 1.5ml cryovials and stored in several freezer locations, determined by the biospecimen type and experiment or use planned. This poses a logistics challenge: accurately accessioning and aliquoting samples into multiple child tubes, while tracking the parent-child relationship between samples and across experiments. The Populus LIMS is used to store all the data regarding the original biospecimen samples collected, and their child tubes. The Populus LIMS also enables storage of biospecimen characteristics such as hemolysis, volume, quality, storage and collection date.

Methods visualization: Collection

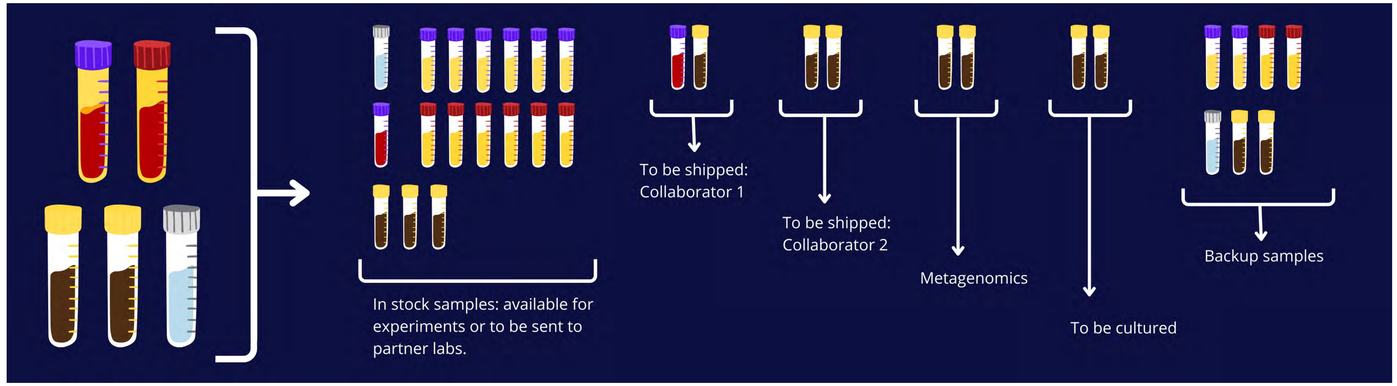

Figure 1. Weekly sample collection and storage distribution

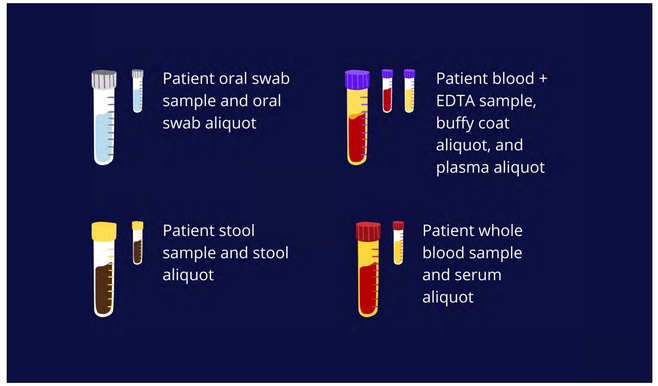

Figure 1a. Legend for Figure 1

Results

Patients	Plasma aliquots	Buffy coat aliquots	Serum aliquots	Oral swab aliquots	Stool aliquots
55	640	214	545	166	567

Figure 2. Total samples registered in custom LIMS system

These samples have been successfully accessioned, aliquoted, and stored, all while maintaining the parent-child relationship to the original sample collected. The custom Populus LIMS serves as an excellent foundation for the DYNAMITE study aims and will enable us to enroll our target of 1000 patients. Sample aliquots are being processed for metagenomics, bacterial culture, and proteomics, with the results from these study procedures collected within the system.

Specimen ID	Source	Type	Quality	Volume	Unit	Condition	Study	Subject	Availability Status	Tray	Tray Location & Contents
DYN_0003_D1_Serum_b	Memorial Hermann Health System Houston	Serum	N/A	500	µL	Normal	Dynamite	DYN_0003	Available	RS_01 (A2)	BH > J > S1 > R2 > DA > Box 1 [1] [RS_01]
DYN_0003_D1_Serum_a	Memorial Hermann Health System Houston	Serum	N/A	500	µL	Normal	Dynamite	DYN_0003	Available	RS_01 (A1)	BH > J > S1 > R2 > DA > Box 1 [1] [RS_01]
DYN_0003_D1_Blood_redCap	Memorial Hermann Health System Houston	Blood (Red Cap)	N/A	0	ml		Dynamite	DYN_0003	Exhausted		

Figure 3. Screenshot of Populus LIMS system

Conclusion

The Populus LIMS is an extremely efficient cloud-based database that maintains parent-child relationship between the depleted original specimens and their respective aliquots. The highly customizable nature of the database provides great opportunity to fine tune the database per the investigator's or research study's demands.

Acknowledgments

We are very grateful to the Populus plus team and their web developers for developing LIMS and customizing it per the needs of the DYNAMITE study.

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2. Center for Infectious Diseases Research, Houston Methodist Research Institute, Houston, TX
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Directed Evolution of Wild Streptomyces towards Antimicrobial Production through Co-Culture with Competitor Pathogens in Microfluidic Droplets

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Background: Soil bacteria *Streptomyces* Spp. are a powerful resource of antibiotics for having provided about two-thirds of all antibiotics. Yet it is evident that there is huge potential of *Streptomyces* to produce new antibiotics as long as their numerous cryptic pathways are evoked. Given the rise and spread of Multi-Drug Resistant pathogens (MDRs), for example, pathogens associated with US hospital-acquired infections, new antibiotics are urgently needed.

Hypothesis: We hypothesize that we can evoke the cryptic pathways of *Streptomyces* to produce new antibiotics via a natural selection approach in a synthetic ecology, where wild *Streptomyces* and clinically relevant pathogens are confined together in a resource-limited micrometer-sized droplet.

Methods: To achieve this goal, we encapsulate single *Streptomyces*^{GFP} cells with a clinically relevant pathogen *Enterococcus faecalis* in a 90-µm droplet. We control the inoculum density of both species so that most of the drops contain 6 cells of *Enterococcus faecalis*, whereas the majority of the drops contain only 1 cell of *Streptomyces*^{GFP}. Following a 2-day incubation, the drops that contain bacteria are interrogated for their fluorescence intensity. The drops that display a strong fluorescence intensity, indicating the prevalence of *Streptomyces*^{GFP} over the population, are sorted, followed by genetic analysis on the winner *Streptomyces*^{GFP} cells as potential antimicrobial producers, and biochemical analysis of the purified molecules as potential antimicrobial leads (Fig. 1).

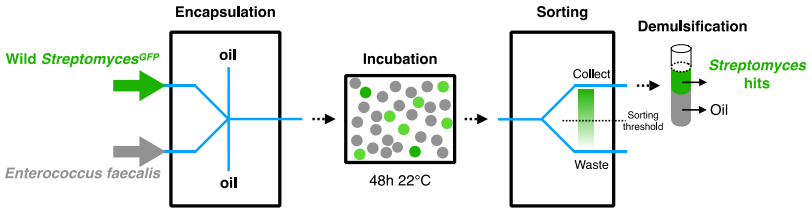


Figure 1. Schematic of the directed evolution of wild *Streptomyces* to antimicrobial production for *Enterococcus faecalis* using drop-based microfluidics.

Results: Most of wild *Streptomyces* have a slow growth rate compared to the *Enterococcus faecalis* strain (days *versus* hours) in lab culture environment. To match the growth rates of *Streptomyces* and *Enterococcus faecalis*, we screened the wild *Streptomyces* isolates in our stock for the fast growers and kept the incubation at a low temperature (~22°C). We successfully inserted the green fluorescent genes in the genome of the fast-growing wild *Streptomyces* and encapsulated the *Streptomyces*^{GFP} with *Enterococcus faecalis* in drops. We observe the growth of *Streptomyces*^{GFP} mono-culture in drops, with characteristic mycelial mats in the early phase, and fragmentation and pigmentation in the late phase. We also observe the growth of *Enterococcus faecalis* mono-culture in drops at a faster growth rate, and the dominance of *Enterococcus faecalis* in the co-culture with wild *Streptomyces*^{GFP} in drops.

Conclusions: We have found and fluorescently labeled the fast-growing wild *Streptomyces* candidates, developed the incubation protocol for synchronized growth of *Streptomyces*^{GFP} and *Enterococcus faecalis* in a micrometer-sized droplet, and demonstrated the successful growth of both bacterial species in the drops. We envision our microfluidics-based assembled ecology as a promising platform for rapid discovery of new antibiotics in an inexpensive and reliable manner.

Acknowledgements: This work is supported by the US National Institutes of Health (5R01AI08071412).

Producing Novel Antimicrobials from Wild *Streptomyces* through Directed Evolution in Microfluidic Droplets

Yizhe Zhang, Xinhao Song, Heer Mehta, Yousif Shamoo* *shamoo@rice.edu*

The Challenge

Soil bacteria *Streptomyces* Spp. are a powerful resource of antibiotics for having provided about two-thirds of all antibiotics. Yet it is evident that there is huge potential of *Streptomyces* to produce new antibiotics as long as their numerous cryptic pathways are evoked. Given the rise and spread of Multi-Drug Resistant pathogens (MDRs), for example, pathogens associated with US hospital-acquired infections, new antibiotics are urgently needed.

Design Strategy

We hypothesize that we can evoke the cryptic pathways of *Streptomyces* to produce new antibiotics via a **natural selection** approach in a synthetic ecology, where wild *Streptomyces* and clinically relevant pathogens are confined together in a resource-limited micrometer-sized droplet.

Approach

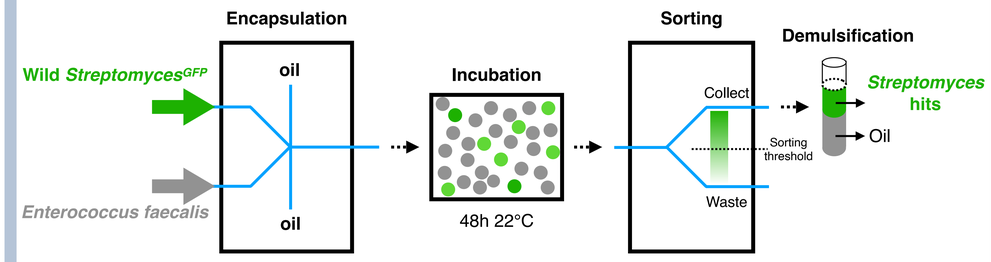
Encapsulating: single *Streptomyces*^{GFP} cells with a clinically relevant pathogen *Enterococcus faecalis* in a 90-μm droplet.

Incubating: microfluidic drops for 2 days to allow for competition between the 2 species.

Sorting: for strong fluorescent drops,

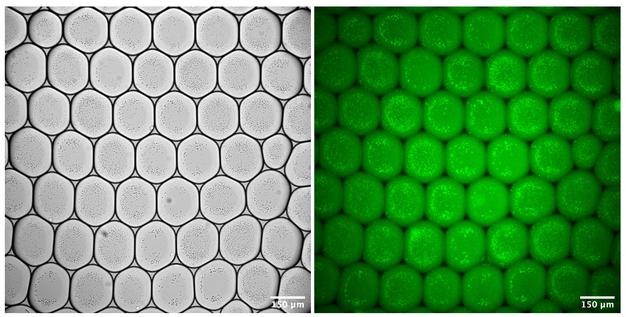
Analyzing: chemical structure of the antimicrobial leads & genetic changes of the producer *Streptomyces*^{GFP} cells.

Directed evolution of wild *Streptomyces* in microfluidic drops

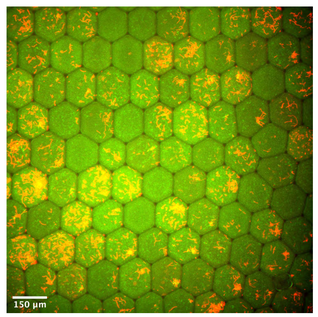


Wild *Streptomyces* and *Enterococcus faecalis* incubated in drops

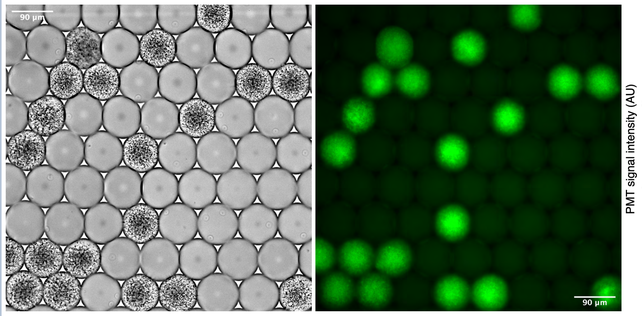
Enterococcus faecalis **mono-culture**



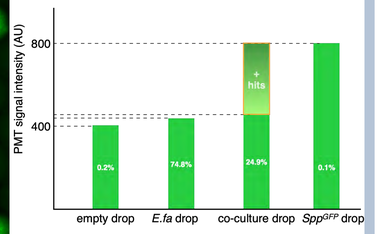
Co-culture



Streptomyces **mono-culture**



Fluorescence detection



Preliminary Results

- Chromosomally labeled the candidate wild *Streptomyces* isolates with GFP
- Synchronized the growth of the 2 species via lowering the incubation temperature (~22°C).
- Established the mono-culture of both species in drops for comparable growth rates
- Observed the dominance of *Enterococcus faecalis* over the wild *Streptomyces*^{GFP} in the co-culture

Future Work

- Generate a library of *Streptomyces*^{GFP} mutants for producer supply
- Screen for the winner *Streptomyces*^{GFP} from the co-culture drops
- Identify the antimicrobial leads and their producers via biochemical and genetic analyses

References

[1] C.A. Arias, B.E. Murray *Nat. Rev. Microbiol.* 10, 266-278 (2012).

[2] L. Mazutis *Nat. Protoc.* 8, 870-891 (2013).

[3] H. Bachmann *Proc. Natl. Acad. Sci. U.S.A.* 110, 14302-14307 (2013).

Acknowledgement

This work is supported by the US National Institute of Allergy and Infectious Diseases (R01AI080714).

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