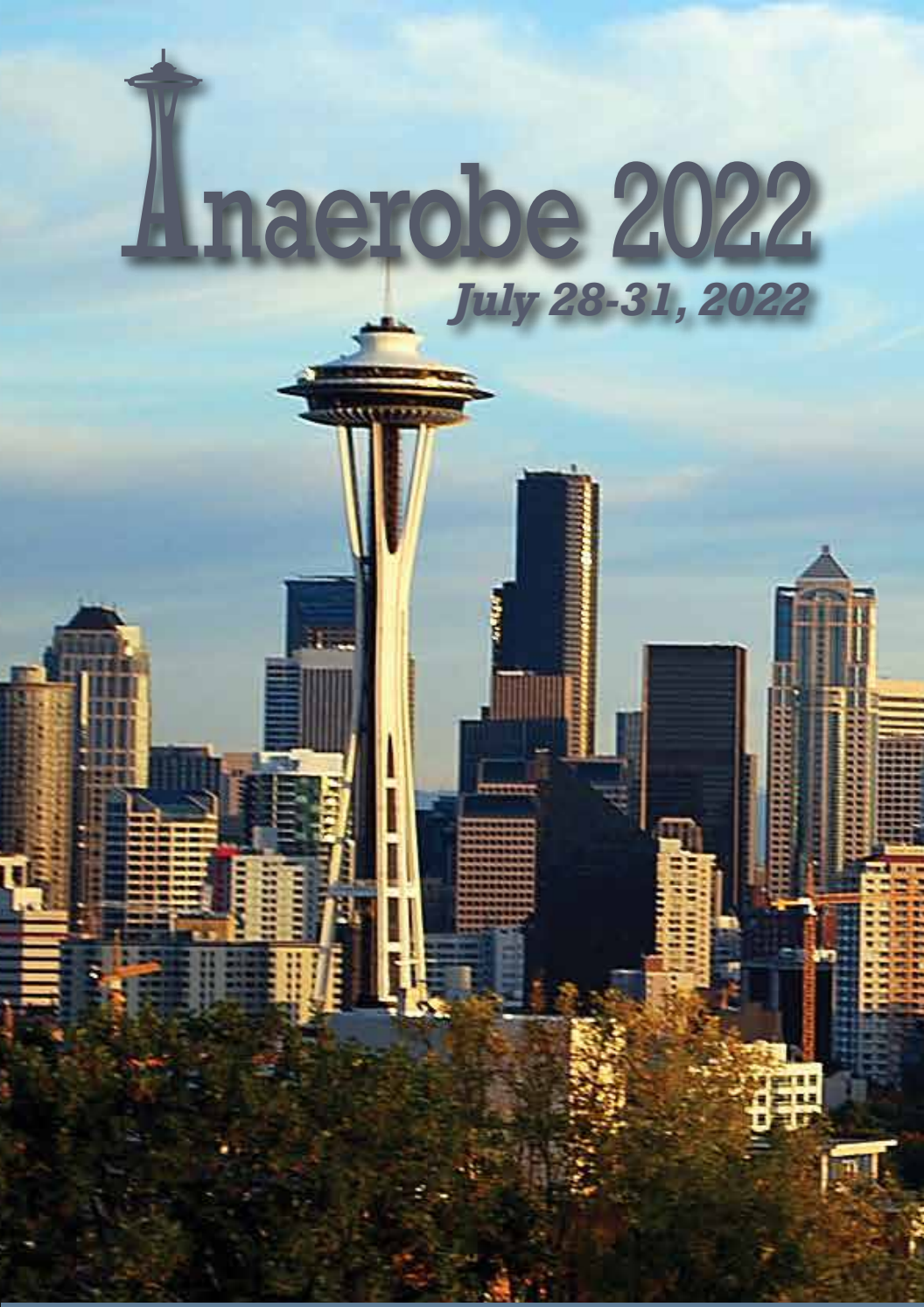


Program and Abstract Book

Anaerobe

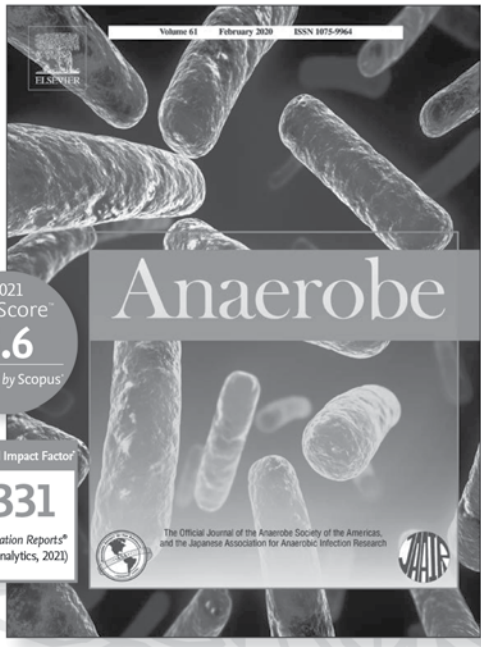
The Official Journal of *the Anaerobe Society of the Americas*, and the *Japanese Association for Anaerobic Infection Research*

Editor-in-Chief

Professor Elisabeth Nagy

University of Szeged, Szeged, Hungary

Anaerobe is essential reading for those who wish to remain at the forefront of discoveries relating to life processes of strictly anaerobes. The journal is multi-disciplinary, and provides a unique forum for those investigating anaerobic organisms that cause infections in humans and animals, as well as anaerobes that play roles in microbiomes or environmental processes. *Anaerobe* publishes reviews, mini reviews, original research articles, notes and case reports.



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Program and Abstract Book

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The 16th Biennial Congress of the Anaerobe Society of the Americas

Dear Colleagues:
Welcome to ***Anaerobe 2022***, The 16th biennial Congress of the Anaerobe Society of the Americas! The past +2 years have been very difficult for all of us, especially those caring for COVID patients, however we were able to scramble to organize a succesful virtual 2020 Congress, which attracted nearly 500 attendees from around the world, participating in many different time zones.

Though still impacted by COVID and other world events, we are convening, this year, in Seattle, in our usual format of in-person oral scientific presentations, posters, and networking. The Program Committee has assembled an exciting program, covering basic science to the impact of anaerobes in non-GI diseases. ***Anaerobe 2022*** again demonstrates the global interest in the field of anaerobic bacteriology, with 172 abstracts submitted for presentation, representing the work of over 630 scientists, spanning 22 countries on six continents.

The *Keynote Address* will be given by Stanley L. Hazen, MD, PhD, the Leonard Krieger Chair of Preventive Cardiology at the Cleveland Clinic in Cleveland, OH. He will discuss his most interesting work in identifying the contributory role of gut microbiota to cardiovascular disease.

At the Congress Banquet, we will take time to remember some of our most distinguished members who we have lost over the past four years, as well as recognizing two others for their valuable contributions to both the clinical and laboratory aspects of anaerobic bacteriology: Dennis Stevens, MD, PhD and Kathryn Bernard, MSc.

We would like to thank the members of the Organizing Committee and the Session Moderators for their assistance in formulating the program. We also would like to show our appreciation to those from foundations, government, and industry—both patrons and exhibitors listed on page v—for the financial support that makes this Congress possible, including grants from the Pfizer, Merck, Acurx, Ferring, Seres, Burroughs Welcome Fund, the National Institutes of Health, the European Society of Clinical Microbiology and Infectious Diseases, and TechLab.

In addition, we are grateful for our continued relationship with Anaerobe Systems for helping organize the Pre-Congress Workshop, Microbiology Educational Services for providing the workshop continuing education accreditation for laboratory scientists, and to our ***Anaerobe*** journal for sponsorship of the Young Investigator's Competition.

Special thanks goes to Susan Bartlett and the Fred Hutchinson Cancer Center for their valuable support, and to Dr. Ronald and Pamela Goldman who, again, brought together the pieces which comprise this meeting.

And, I hope you have time to explore and enjoy our Emerald City of Seattle.

David N. Fredricks, MD
President, Anaerobe Society of the Americas

July 28-31

About the Anaerobe Society

Founded in 1992, the Anaerobe Society of the Americas, a non-profit foundation, serves as a forum for those interested in anaerobes, anaerobic infections, and related matters. The Society aims: (1) to stimulate interest in anaerobes and to encourage interchange among anaerobists from all disciplines, including medical, dental, veterinary, environmental, and basic sciences; (2) to bring together investigators, clinicians, and laboratory scientists interested in anaerobic infections for formal and informal meetings; (3) to review and assess new advances in the field; (4) to discuss areas of controversy; and (5) to mark future directions.

There are four levels of membership: Doctoral, Non-Doctoral, Verified Student, and Retired. Details and application form are available on our web site: *www.anaerobe.org*.

Anaerobe Society Congresses

This is the 16th biennial Anaerobe Society Congess.
Past Anaerobe Society sponsored programs were:

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For Additional Information

About the Anaerobe Society or ***Anaerobe 2022***

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Patrons & Exhibitors

Anaerobe Society of the Americas gratefully acknowledges the following organizations for their generous support of the 16th biennial Congress.

Independent educational grants, without involvement or influence, were received from:

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Keynote Speaker

Stanley L. Hazen, MD, PhD

Dr. Hazen is the Chair of the Department of Cardiovascular & Metabolic Sciences, Professor in the Department of Molecular Medicine, and the Leonard Krieger Chair of Preventive Cardiology at the Cleveland Clinic in Cleveland, OH. He received his BA in Biochemistry, MD, and Ph. in Biophysical Chemistry & Molecular Biology from Washington University in St. Louis, MO. He currently serves as Associate Editor of the *European Heart Journal* and the *Journal of Lipid Research*, as well as reviewer for several journals.

As a biophysical chemist and physician, Dr. Hazen has devoted his professional career to the treatment and investigation of pathways contributing to atherosclerotic heart disease and other inflammatory disorders. He uses mass spectrometry as a discovery platform, interrogating human clinical samples at the chemical level to reveal processes that occur *in vivo*. Then using multidisciplinary approaches, studies are designed to "reverse engineer" clinically-relevant processes, aiming to understand their origins at the structural, genetic, molecular, cellular, and animal model level. Findings discovered at the bench are used to instruct human clinical investigations, which both serve to validate the physiological relevance of findings, and further investigate mechanistic understandings.

His multidisciplinary teams use this iterative approach on multiple research programs. One such program involves the discovery of a contributory role of gut microbiota to cardiovascular disease (CVD). Using untargeted metabolomics as a discovery platform, their studies revealed how specific dietary nutrients, gut microbes, and specific microbial enzyme pathways produce compounds (e.g. trimethylamine N-oxide, TMAO) that directly contribute to the development of both renal functional decline and heart failure development. They have pursued therapeutic efforts to target the gut microbiome and reported the first development of non-lethal small molecule inhibitors of the microbial TMAO pathway for the treatment of both diet induced atherosclerosis, and platelet hyperreactivity and enhanced thrombosis potential. These studies logically followed their recent demonstration that the TMAO gut microbial pathway is mechanistically linked to thrombosis potential and alteration in platelet function via changes in stimulus dependent calcium signaling. More recently, they have used a similar untargeted metabolomics approach, coupled with animal model and microbial engineering/transplantation studies, to discover a new gut microbiota dependent pathway linked to CVD pathogenesis in type 2 diabetes. Their new studies show the gut microbiome dependent metabolite, PAGln (phenylacetylglutamine), impacts host physiology *via* acting as an allosteric modulator of adrenergic receptors.

He also takes great pride in mentoring the next generation of both basic scientists and physician investigators at all levels. Many prior members of the Hazen lab have moved on to successful careers in clinical and academic medicine, as well as biomedical/industry related research positions.

Lifetime Achievement Award

Dennis Stevens, MD, PhD

Dr. Dennis L. Stevens is a world-renowned clinician-scientist at the VA Medical Center in Boise, ID. As an infectious disease specialist, he has taken care of the clinical needs of Veterans for over 5 decades. As a researcher, he is an internationally-recognized authority on the pathogenesis and treatment of life-threatening necrotizing Gram positive soft tissue infections, including those caused by various clostridial species such as *C. perfringens, C. septicum*, and *C. sordellii*.

Through his own investigations, he has unraveled key host-pathogen interactions and has provided clear and definitive scientific rationale for improved treatments. His discoveries prompted a significant paradigm shift in clinical management of cases in that inhibition of toxin production and/or activity has become an important clinical goal. Collectively, his work has formed the basis for the current recommended treatment of severe soft tissue infections due to *Streptococcus pyogenes, Staphylococcus aureus,* and histotoxic clostridial species. His research exemplifies true translational medicine, in which his astute clinical observations led to sound basic science investigations and seminal discoveries that both advanced our knowledge of biology and improved clinical management of these common, and often devastating, infections.

His earliest basic science publication *(JID,* 1988) elegantly demonstrated in a mouse myonecrosis model that clindamycin, a protein synthesis inhibitor antibiotic, was strikingly more effective than penicillin in preventing mortality from *C. perfringens* and Group A *Streptococcal* disease (GAS). He subsequently (*JID,* 1993) provided the first molecular explanation for this phenomenon known as the "Eagle effect," showing that penicillin's killing of GAS was diminished at high bacterial concentrations due to loss of critical streptococcal penicillin-binding proteins during stationary-phase growth. He further showed clindamycin was superior, largely due to its ability to block bacterial toxin production in streptococcus and clostridial species. These studies fundamentally improved treatment by providing basic science rationale supporting a critical change in medical practice.

His publications are numerous, including a comprehensive review on Necrotizing Soft Tissue Infections for the *New England Journal of Medicine,* monographs for the NIH e-book series, chapters on pathogenesis and treatment of invasive infections for major medical textbooks, and over 300 journal articles. He is Chair of the IDSA's Guidelines Committee for the Treatment of Skin and Soft Tissue Infections, a consultant to WHO and NIH, and section editor for *Current Opinions in Infectious Disease.* He received the IDSA's *Society Citation* in 2000 and the *VA Society of Practioners in Infectious Disease Award* in 2018

Dr. Stevens, with his scientist-wife Dr. Amy Bryant, has maintained VA and NIH research support throughout his career, including a prestigious 5-year, $10 million NIH Center of Biomedical Research Excellence (COBRE) grant to establish a Center of Excellence in Emerging/Reemerging Infectious Diseases at the Boise VA.

Lifetime Achievement Award

Kathryn Bernard, MSc

Kathryn Bernard is an internationally-recognized subject matter expert in the characterization of rare, difficult-to identify or novel bacteria, which cause disease in humans. This was primarily done during her nearly 42-year career as a microbiologist with Canada's National Microbiology Laboratory (NML–Public Health Agency of Canada) in Winnipeg, where she served as head of the Special Bacteriology Unit (retired March 2021).

Kathy received her BSc from the University of Windsor (Windsor ON) and her MSc from McGill University (Montreal QC). She is accredited through the Academic and Research Microbiologist (ARM) from the Canadian College of Microbiology (CCM, no US equivalent) and is an Adjunct Professor/Lecturer in the Department of Medical Microbiology at the University of Manitoba.

Besides being a frequent reviewer for high-impact journals, Kathy has been lead or co-author of 140 peer-reviewed publications, 110 papers presented at conferences, as well as book chapters, monographs, and anaerobic clinical guidelines. Her work has been cited by nearly 5,000 publications, including her work with many such anaerobic species as *Clostridium neonatale*, *Clostridium lavalense, Propionibacterium australiense, Clostridium sordellii, Corynebacterium hindlerae, Corynebacterium xerosis, Pseudoxanthomonas winnipegensis*, and *Lawsonella clevelandensis*, and *Gardnerella vaginalis*. She also has been a frequently-invited guest speaker for microbiology organizations throughout Canada and the U.S., as well as educating lay audiences.

She has received three national awards for microbiology in Canada: the *Commemorative Medal for the Golden Jubilee of her Majesty Queen Elizabeth II* (2002), the *John G. Fitzgerald Award as Outstanding Microbiologist* from the Canadian Association of Clinical Microbiology and Infectious Diseases (2015) and the *Distinguished Microbiologist Award,* from the Canadian College of Microbiology (2016). She has recently been elected to the American Academy of Microbiology (2022).

Kathy even has a nonspore-forming, nonmotile, facultative anaerobic, gram-positive coccobacilli, *Trueperella bernardiae*, named in her honor. Though retired, she continues to work in the field through her KA Bernard Consultancy in Bacteriology and Public Health.

In Memoriam

Sidney Finegold, MD—ASA Founding President

Dr. Finegold was "Mr. Anaerobe." For more than 50 years, he studied every aspect of anaerobic bacteriology and its clinical relevance, and his work changed the practice of medicine throughout the world by demonstrating the importance of anaerobes in common infections. His work is well-recognized in both the fields of microbiology and infectious diseases.

Dr. Finegold was Chief of the Chest and Infectious Disease sections and Chief of the Research Service at the VA Medical Center in West Los Angeles, CA, as well as Professor of Medicine and Microbiology, Immunology, and Molecular Genetics at UCLA.

Beginning his career studying gut flora, he investigated the role of anaerobes in the blind loop syndrome and the effects of diet on fecal flora. After verifying the work of Dr. John Bartlett and Dr. Sherwood Gorbach on *Clostridium difficile* as the agent of pseudomembranous colitis, his research team developed a selective medium for *C. difficile* and noted environmental contamination with this organism in a hospital setting.

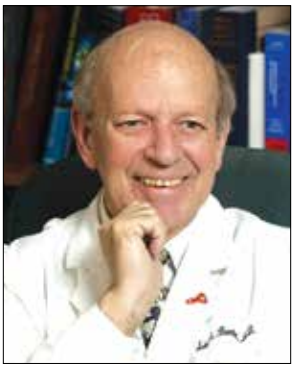
Other research accomplishments included the importance of anaerobes as pathogens relating to pulmonary, intra-abdominal, and skin and skin-structure infections; development of laboratory techniques for the isolation and identification of anaerobes; standardization of anaerobic antimicrobial susceptibility testing procedures; studies on antimicrobial therapy of anaerobic infections; the first descriptions of more than two dozen new genera or species; and the combined use of biochemical and molecular techniques for the identification of anaerobes.

He was Founding President of the Anaerobe Society and was Editor-in-Chief of its journal, *Anaerobe,* for 10 years. Dr. Finegold was Past President of the Infectious Diseases Society of America, the Society of Intestinal Microbial Ecology and Disease, and the VA Society of Practitioners in Infectious Disease and has served as Editor of Reviews of *Infectious Diseases and Clinical Infectious Diseases* for 10 years. He was a Master of the American College of Physicians and a Fellow of the American Academy of Microbiology.

His career is well-documented with over 800 publications, including almost 400 research papers, more than 100 review articles, 39 books authored or edited, and almost 200 book chapters. Most notable is the *Wadsworth Anaerobic Bacteriology Manual* and *Anaerobic Bacteria in Human Disease.* His numerous awards included the *Veterans Administration Middleton Award for Biomedical Research, Wadsworth First Annual Lifetime Achievement Award,* ASM's *Hoechst Marion Roussel* and Becton *Dickinson Awards,* IDSA's *Bristol and Aventis Pharmaceuticals Awards,* Finland's *Medal of Helsinki,* the first *Outstanding Achievement Award* of the Infectious Disease Association of California, and the *Lifetime Achievement Award* of the Anaerobe Society of the Americas.

However, his most important impact has been his numerous "children," who have trained with him and have gone on to their own outstanding careers in microbiology, infectious diseases, pulmonology, gynecology, surgery, and veterinary medicine. Dr. Finegold passed away in August 2018, at age 97.

In Memoriam



John G. Bartlett, MD.

Dr. Bartlett was among the most esteemed figures in the field of infectious diseases.

He received his MD from Upstate Medical Center in Syrcuse, NY, and trained in internal medicine at the Pete Bent Hospital in Boston, MA and the University of Alabama in Birmongton, AL. He completed his fellowship training in infectious disease at the University of California, Los Angeles (UCLA), under the tutelage of Dr. Sydney Finegold.

He served as a faculty member at the UCLA School of Medicine and Tufts University School of Medicine in Boston, MA, as well as Chief of Staff for Research at Boston VA Hospital. He, then, moved onto Johns Hopkins University in Baltimore, MD, where he served as Chief of the Division of Infectious Diseases for 26 years, 1980-2006.

His major research interests were anaerobic infections, pathogenic mechanisms of *Bacteroides fragilis;* anaerobic pulmonary infections; and *Clostridium difficile*-associated colitis. Most notable was his work with Dr. Sherwood Gorbach on identifying *Clostridium difficile* as the agent of pseudomembranous colitis, published in the *New England Journal of Medicine* in 1978. The Anaerobe Society celebrated the 30th anniversary of this publication at the *Anaerobe 2008* Congress, where both Drs. Bartlett and Gorbach were honored with *Lifetime Achievement Awards.* His later work addressed HIV/AIDS, managed care of HIV patients, and bioterrorism.

During his career, Dr. Bartlett authored over 500 articles and reviews in peer-reviewed journals, more than 280 book chapters, and 67 editions of 18 books. He served on editorial boards for 19 medical journals. Though he officially retired in 2014, he continued to write, edit, and speak on infectious disease topics.

Dr. Barlett was a member of the Institue of Medicine, a Master of the American College of Physicians, and President of the Infectious Disease Society of America (IDSA). His numerous honors include the IDSA's *Alexander Fleming* and *Kass Awards,* and the *Finland Award* from the National Foundation for Infectious Diseases. However, his interests extended beyond medicine. One of his most beloved pastimes was painting.

Reflecting on his career, Dr. Bartlett admitted, "A lot of my decisions were made without the idea that this is a really important part of my life or my career. But, of course, you often don't know that at the time." When asked what advice he would give to the next generation of physician scientists, he offered, "Follow your passion, follow your gut—it might just lead you to the next big thing."

Dr. Bartlett passed away in January 2021, at age 83.

In Memoriam

J. Glenn Songer, PhD.

Dr. Songer was a professor and researcher in Veterinary Microbiology for 37 years at Iowa State University and University of Arizona. He was particularly known for his work researching clostridial diseases in animals. He authored 71 scientific publications, co-authored 3 textbooks, and had 857 citations. His books included *Veterinary Microbiology: Bacterial and Fungal Agents of Animal Diseases, Pathogenesis of Bacterial Infections in Animals,* and *Clostridial Diseases of Animals.* He was Fellow of the American Academy of Microbiology and Diplomate of the American College of Veterinary Microbiology.

He also was active in the Anaerobe Society, serving on the board for 9 years including positions of Secretary and Vice President. In addition, he was a former Editor-in Chief of the *Anaerobe* journal. As a hobbyist knifemaker, he forged special knives as outgoing gifts for ASA Presidents. He participated in several international research collaborations with Anaerobe Society members. leading to publications. Dr. Songer passed away in July, 2021 at age 71.

Hebe Bianchini, PharmD, MD

Dr. Bianchini of Argentina attended the first Anaerobe Congress in 1992, where she presented *Broth Disk Elution Method for Anaerobic Bacteria: A Collaborative Study to Assess Its Reliability for Clinical Purposes,* later published in the *Anaerobe* journal. Afterwards, she became an early ambassador of anaerobic microbiology throughout South America.

Dr. Bianchini was a Biochemist, Pharmacist, Doctor, and Professor of Microbiology at the University of Buenos Aires. She belonged to the group that developed the new generation of Microbial Diagnosis in the 70's in Argentina. Her career was focused on strengthening the knowledge of infections caused by anaerobic bacteria. She was President of the Argentine Association of Microbiology (AAM) (1991-1995). She created the AAM Anaerobic Bacteria Subcommittee, with the aim of transferring her knowledge on microbial diagnosis in relation to anaerobes. Hebe was an extraordinary woman, whose research opened doors for many microbiologists, and her legacy lives on in many hospitals. Her work positioned women in a prestigious position in microbiology in Argentina.

In 1998, she hosted the *4th Biennial Anaerobe Congress* in Buenos Aires, which was the largest attended meeting of the Anaerobe Society attracting attendees from throughout the hemisphere and beyond. Dr. Bianchini passed away in June, 2022 at age 88.

July 28-31

Anaerobe 2022—The 16th biennial Congress of the Anaerobe Society of the Americas—provides the forum for vigorous discussions of both the clinical and microbiological aspects of anaerobic infections, their diagnosis, and their therapy among medical practitioners, researchers, laboratory scientists, and industrial representatives.

Curricular Goals & Objectives

Provide information on the latest developments in the field of anaerobic research, including the role of anaerobes in human diseases, the epidemiology of anaerobic infections, and potential prevention strategies.

Provide recommendations in the diagnosis, screening, and treatment of anaerobic infections, including new laboratory techniques, utilization of antibiotics, and potential of probiotics.

Provide an understanding for better utilization of the microbiology lab into the delivery of patient care.

Accreditation & Certificates of Attendance

This activity has been planned and implemented in accordance with the Essentials Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME).

Pre-Congress Workshops: Microbiology Educational Services is accredited by the California Department of Health Services to provide continuing education for clinical laboratory scientists and designates 7.0 continuing education contact hours upon completion of a workshop. Clinical laboratory scientists should claim only those hours of credit that they actually spent in the educational activity.

Congress: No Continuing Education Units will be issued for this Congress. Attendees may request Certificates of Attendance, free of charge, on the Registration Form or upon registering, which will be emailed after Congress evaluations are processed.

Disclosures

Disclosures of relevant financial relationships by all session participants are provided on pages xvii-xviii.

Evaluation Forms

Please complete the Evaluation Form at the end of the Congress and return to the Registration Desk. If you wish to receive a Cetificate of Attendance, check the box on you completed Evaluation Form and be sure to include you email address, as certificates will be sent *via* email.

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The following presenters have information to disclose as follows:

Michael C. Abt, PhD	Merck (G)
Frederick J. Angulo, DVM, PhD	Pfizer Vaccines (E, O)
Jennifer M. Auchtung, PhD	Synbiotic Health (E)
Ken Blount, Ph.D.	Ferring Pharmaceuticals /Rebiotix (E) Clinical Trial Data
Susan Bullman, PhD	BioMx (C), GlaxoSmithKline (C)
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Laura M. Cox, Ph.D.	Anaerobe Systems (E)
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Niokhor Dione, PhD	Animal Microbiome Analytics (E, O)

Participant Disclosurers, continued

Christopher Ford, PhD	Seres (E)
David N. Fredricks, PhD	Becton-Dickinson (R)
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Thursday, July 28

0800

WORKSHOPS & CONGRESS REGISTRATION OPENS

0900-1700

ANAEROBIC IDENTIFICATION & SUSCEPTABLITY WORKSHOP

Diane M. Citron

Mike Cox

0900-1700

EXAMINING ANAEROBES IN THE MICROBIOME: METAGENOMIC AND CULTURE APPROACHES

Laura M. Cox, Ph.D.

Casey M. Theriot, Ph.D

Anna M. Seekatz, Ph.D.

1800

PRE CONGRESS MEET & GREET SOCIAL

Hyatt Regency Hotel, 7th Floor Foyer

Friday, July 29

0700-0820 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS

0820

WELCOME REMARKS

David N. Fredricks, MD, ASA President

0830-0930

SESSION I: KEYNOTE PRESENTATION

Moderator: David N. Fredricks, MD

SI-1 Gut Microbial Metabolism and Cardiovascular Disease
Stanley L. Hazen, MD, PhD

0930-0945 BREAK / INDUSTRY EXHIBITS

0945-1145

SESSION II: OUT OF THE BOX: IMPACT OF GUT MICROBES ON NON-GI DISEASE

Moderator: Cynthia Sears, MD

SII-1 The Microbiome and Cancer Immunotherapy
Fyza Shaikh, MD, PhD

SII-2 Spingholipids as Modulators of Microbiome Function
Elizabeth L. Johnson, PhD

SII-3 Specific Gut Bacteria Regulate Innate Immune Functions in the Brain of Aged Mice Related to Alzheimer's Disease
Caroline Wasén, PhD

SII-4 *Clostridium perfringens* Epsilon Toxin as a Possible Environmental Cause of Multiple Sclerosis in Humans
Jennifer Linden, PhD

0945

SAT-1 Satellite Symposium: Introducing the Stratus, Your Solution to Anaerobic Bacterial Growth Curves
Cerillo

1145-1230 LUNCH / INDUSTRY EXHIBITS

1230-1330 POSTER SESSION I / INDUSTRY EXHIBITS

1330-1515

SESSION III: FUSOBACTERIA

Moderator: Yiping Han, PhD

SIII-1 Mechanistic Insight of *Fusobacterium nucleatum* in Colorectal Cancer
Yiping W. Han, PhD

SIII-2 Experimental Approaches to Identify Virulence Determinants in *Fusobacterium nucleatum*
Hung Ton-That, PhD

SIII-3 Fusobacteria-Dominant Colorectal Cancer Biofilms are Associated with High Levels of the Polyamine N1, N12-Diacetylspermine
Julia Drewes, PhD

SIII-4 *Fusobacterium nucleatum* Subspecies Differ in Biofilm Forming Ability *in vitro*
Maria Muchova

Friday, July 29

SESSION IV: METHODOLOGY & EPIDEMIOLOGY

Moderator: Karen Carroll, MD

SIV-1 Understanding the Roles of TcdE and TcdL in Toxin Secretion
Shannon Kordus, PhD

SIV-2 Identification of a Bile Acid-Binding Transcription Regulator in *Clostridioides difficile* Using Chemical Proteomics
Emily Forster

SIV-3 Natural Mutation Identified in CdtR of Clinical *Clostridioides difficile* Isolate Attenuates Virulence in Mice
Qiwen Dong, PhD

SIV-4 FliW and CsrA Govern Flagellin (FliC) Synthesis and Play Pleiotropic Roles in Virulence and Physiology of *Clostridioides difficile* R20291
Xingmin Sun, PhD

SIV-5 Comparative Genomic Analysis of *Clostridioides difficile* Strain Restriction Endonuclease Analysis Group Y across Four Decades
Andrew Skinner, MD

SIV-6 Anaerobic Microbiota Facilitate Pathogen Access to the Airway Epithelium in a Novel Co-Culture Model of Colonization
Ryan Hunter, PhD

1330-1515

1515-1530 BREAK / INDUSTRY EXHIBITS

SESSION V: BUGS AS DRUGS: ENGINEERED MICROBIAL COMMUNITIES & FECAL MICROBIOTA TRANSPLANT

Moderator: Vincent Young, MD

SV-1 Current State of Knowledge: Fecal Microbiota Transplant for Fecal MDRO Colonization
Jennie H. Kwon, DO, MSCI

SV-2 Consistent Microbiome and Bile Acid Restoration Across Three Clinical Trials of RBX2660 for Recurrent *Clostridiodes difficile* Infection: A Combined Analysis
Ken F. Blount, PhD

SV-3 Impact of SER-109: An Investigational Microbiome Therapeutic, on Stool Fatty Acid and Bile Acid Metabolites in a Phase 3 Randomized Trial (ECOSPOR III) for Treatment of Recurrent *Clostridioides difficile* Infection (rCDI)
Christopher Ford, PhD

SV-4 Efficacy and Safety of SER-109: An Investigational Microbiome Therapeutic for Treatment of Recurrent *Clostridioides difficile* Infection (rCDI): A Phase 3 Double-Blind, Randomized Trial (ECOSPOR III)
Thomas Louie, MD

1530-1730

Friday, July 29

1530-1730

SESSION VI: THE ORAL MICROBIOME AND HUMAN HEALTH

Moderator: Jeff S. McLean, PhD

SVI-1 Precision-Guided Antimicrobial Therapy for Targeted Modulation of Human Microbiome
Wenyuan Shi, PhD

SVI-2 Anaerobic *pas de deux*: Introducing *Parvimonas micra* Genetics and Its Interspecies and Host Interactions
Justin L. Merritt, PhD

SVI-3 Experimental Gingivitis: Lessons Learned from Chemokine Multi-Plex and Bacterial Sequence Analysis
Richard P. Darveau, PhD

SVI-4 A Genomic Portrait Reveals the Genetic Plasticity and Evolutionary Adaptation of *Porphyromonas gingivalis*
Mohamed Abdelbary, PhD

SVI-5 A Revised Taxonomy of the Genus Treponema Delineating Commensal and Pathogenic Organisms
Paul Lawson, PhD

1730-1800 ASA BUSINESS MEETING

1800-1900 WINE & CHEESE RECEPTION

Saturday, July 30

0700-0800 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS

0800-0945

SESSION VII: *FUNCTIONAL ROLES OF ANAEROBES IN THE GASTROINTESTINAL TRACT*

Moderator: David N. Fredricks, MD

SVII-1 Glycan Scavenging by Human Gut Bacteria
Nicole M. Koropatkin, PhD

SVII-2 Microbial Bile Acid Conjugation as a New Mechanism of Host-Microbiome Crosstalk
Robert A. Quinn, PhD

SVII-3 Characterization of Rare Lachnospiraceae
Anna Seekatz, PhD

SVII-4 Diversity, Phylogeny, and Antimicrobial Nature of a Bile Acid Conjugation Within the Lachnospiraceae
Douglas Guzior

0945-1000 BREAK / INDUSTRY EXHIBITS

Saturday, July 30

SESSION VIII: *CLOSTRIDIOIDES DIFFICILE*: MANAGEMENT UPDATE

Moderator: Vincent Young, MD

SVIII-1 Diagnosis of ***C. difficile*** Infection: NAAT *vs*. Other Algorithms, Ultrasensitive Toxin Testing
Karen Carroll, MD

SVIII-2 Treatment and Prevention: IDSA/SHEA Guideline Update
Stuart Johnson, MD

SVIII-3 Effect of the COVID-19 Pandemic on Rates of *Clostridioides difficile* Infection in One VA Hospital and Correlation with Rates in the National VA Healthcare System
Lorinda Wright, PhD

SVIII-4 Update on Ibezapolstat Clinical Trial Development
Kevin Garey, PharmD

SVIII-5 Impact of Misdiagnosis of *Clostridioides difficile* Infections (CDI) by Standard-of-Care Specimen Collection and Testing on Estimates of Hospitalized CDI Incidence Among Adults in Louisville, Kentucky, 2019-2020
Frederick J. Angulo, DVM, PhD

1000-1205

1205-1345 LUNCH / INDUSTRY EXHIBITS

1230-1330 YOUNG INVESTIGATOR'S PRESENTATIONS

SESSION IX: BREAKOUT LUNCH SESSION

Moderator: Yiping W. Han, PhD; Presenter: Cynthia L. Sears, MD

SIX-1 Meet the Editors: Tips on Publishing in Peer-Reviewed Journals

1230-1330

SESSION X: BASIC SCIENCE OF *CLOSTRIDIOIDES DIFFICILE* INFECTION

Moderator: Stuart Johnson, MD

SX-1 Phenotypic Heterogeneity in *Clostridioides difficile*
Rita Tamayo, PhD

SX-2 Immune-Microbiota Interactions in Defense Against *Clostridioides difficile*
Michael C. Abt, PhD

SX-3 Impact of the *C. difficile* Small Acid Soluble Proteins of Spore Physiology
Joseph A. Sorg, PhD

SX-4 Sneak Attacks: Host Defense Peptides Enhance Antibiotic Efficacy Against *C. difficile* Without Triggering Spore Formation
Erin Purcell, PhD

SX-5 Colonization of Hamsters with Nontoxigenic *Clostridiodes difficile* REA Type M3 (M3), Following Treatment with Fidaxomicin or Vancomycin
Susan Sambol

SX-6 Distinct Single-Cell Growth Dynamics of *Clostridioides difficile* Clade 5 Strains Revealed by Anaerobic Time-Lapse Microscopy
John Ribis, PhD

1345-1615

Saturday, July 30

1345-1615

SESSION XI: OPTIMIZING ANAEROBIC CULTIVATION

Moderator: Sujatha Srinivasan, PhD

SXI-1 Revealing the Hidden Lifestyle of an Ultra-Small Saccharibacteria
Batbileg Bor, PhD

SXI-2 Human Microbiota Diversity through Culture Enriched Metagenomics
Michael G. Surette, PhD

SXI-3 Anaerobic Cultivation and Strain-Banking from the Human Gastrointestinal Tract
Anne Neville, PhD

SXI-4 Enhancing the Abundance of Understudied Fastidious Species Within Oral Microcosms
Madeline Krieger, PhD

SXI-5 Automated Analysis of Microbial Growth Reveals Phenotypic Diversity of *Clostridioides difficile*
Firas Midani, PhD

SXI-6 *Pseudomonas aeruginosa* Isolates from Chronic Rhinosinusitis Develop Virulence Under Anaerobic Conditions
Do-Yeon Cho, MD

1615-1630 **BREAK / INDUSTRY EXHIBITS**

1630-1730

SESSION XII: *CLOSTRIDIOIDES DIFFICILE* HOT TOPICS

Moderator: Daniel Paredes-Sabja, PhD

SXII-1 Taurine Conjugated Bile Acids Ameliorate Apoptosis Caused by *Clostridioides difficile* Toxins
Casey M. Theriot, PhD

SXII-2 Olfactomedin-4 Exacerbates *Clostridioides difficile* Infection-Induced Mortality in Mice
Shinsmon Jose, PhD

SXII-3 Sex-Mediated Differences in Murine *Clostridioides difficile* Infection Outcome
Ernesto Abel-Santos, PhD

SXII-4 The Difficile Genomics Sequencing and Typing Service
Trefor Morris, MSc

1630-1730

SESSION XIII: GENETIC MANIPULATION OF ANAEROBES: STRATEGIES AND SUCCESSES

Moderator: Sarah A. Kuehne, PhD

SXIII-1 In Pursuit of a Fusobacterium 'Pan-genetic' System
Daniel Slade, PhD

SXIII-2 Systematic Design of Genetic Systems for Non-Model Organisms to Advance Functional Interrogations of the Human Microbiota
Christopher Johnston, PhD

1900 **BANQUET RECEPTION / DINNER / AWARDS**

Sunday, July 31

0700-0800 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS

SESSION XIV: EMERGING ANAEROBES AND DISEASE ASSOCIATIONS

Moderator: Audrey N. Schuetz, MD

SXIV-1 The *Veillonellaceae*: An Emerging Pathogenic Genus with Changing Susceptibility Patterns
Ellie J.C. Goldstein, MD

SXIV-2 Novel Anaerobes in the Reproductive Tract
Sujatha Srinivasan, PhD

SXIV-3 Mechanisms of Virulence and Protection in *Paeniclostridium sordellii* Infection
Sarah Bernard, MS

SXIV-4 *Sneathia vaginalis*: An Emerging Pathogen of Pregnancy and Its Pore-Forming Toxin
Kimberly Jefferson, PhD

SXIV-5 *Gardnerella* Diversity and Ecology in Pregnancy and Preterm Birth
Hannah Berman

SXIV-6 Glycogen Break Down by Vaginal Bacteria
Elliot Lee, EdM

0800-1000

1000-1015 BREAK / INDUSTRY EXHIBITS

SESSION XV: ANAEROBES IN LOW BIOMASS ENVIRONMENTS

Moderator: David N. Fredricks, MD

SXV-1 Intro to the Challenge: The Placental Microbiome?
David N. Fredricks, MD

SXV-2 Fallopian Tube Microbiome Analysis: Potential Pitfalls
Bo Yu, MD, MS

Point-Counterpoint Debate: Anaerobes in the Lung: Cystic Fibrosis

SXV-3 —Anaerobes as Pathogens
Michael G. Surette, PhD

SXV-4 —Anaerobes as Commensals and Transients
Pradeep Singh, MD

—Panel Discussion

SXV-5 Mapping the Intratumoral Microbiota in Human Oral and Colorectal Cancers
Susan Bullman, PhD

1015-1215

Sunday, July 31

1015-1215

SESSION XVI: ONE HEALTH: ANAEROBES IN HUMANS, ANIMALS, AND THE ENVIRONMENT

Moderator: Francisco A. Uzal, DVM, PhD

SXVI-1 New insights into Sporulation and Enterotoxin Production by *Clostridium perfringens* Type F
Bruce A. McClane, PhD

SXVI-2 Genomic and Evolutionary Insights into *C. difficile:* The Quintessential One Health Pathogen
Daniel R. Knight, PhD

SXVI-3 Microbiome Therapy in Companion Animals: Strategies for the Creation of Anaerobic Microbial Complexes
Niokhor Dione, PhD

SXVI-4 Evaluating the Role of Pore Formation in *Clostridium perfringens* Enterotoxin Permeability Effects
Archana Shrestha, PhD

1125-1315 LUNCH / INDUSTRY EXHIBITS

1315-1415 POSTER SESSION II / INDUSTRY EXHIBITS

1415-1600

SESSION XVII: CLINICAL INFECTIOUS DISEASE: ANAEROBE INFECTIONS

Sponsor: European Society of Clinical Microbiology and Infectious Diseases
Moderator: Vincent Young, MD

SXVII-1 Increased Variety of Actinomyces Infections
Eija Könönen, DDS, PhD

SXVII-2 Diabetic Wound Infections
Elizabeth A. Grice, PhD

SXVII-3 Real Time Surveillance of Antimicrobial Resistance in Anaerobic Bacteria
Trefor Morris, MSc

SXVII-4 Eucast Breakpoints and Methods for Susceptibility Testing of Anaerobic Bacteria
Trefor Morris, MSc

1415-1600

SESSION XVIII: MODEL SYSTEMS TO ELUCIDATE THE BIOLOGY OF ANAEROBES

Moderator: Aimee Shen, PhD

SXVIII-1 Bioreactors: A Versatile Tool for Characterizing Microbial Community Disruption and Recovery
Jennifer M. Auchtung, PhD

SXVIII-2 Determinants of Biofilm Formation in the Anaerobe Gut Symbiont *Bacteroides thetaiotaomicron*
Jean-Marc Ghigo, PhD

SXVIII-3 Synbiotic Delivery of Human Microbiota and Paired Fiber Improves Post-Antibiotic Resilience in Mice in Sex-Dependent Ways
Steve Lindemann, PhD

1600 CLOSING REMARKS / CONGRESS ADJOURNS

Oral Abstract Contents

This abstract book is divided according to the Congress sessions. The table below identifies the pages pertaining to each session in the contents and among the abstracts.

Poster Abstract Contents

Friday, July 29 — Keynote Session

0830-0930 Session I: Keynote Presentation

*—Indicates Presenter

GUT MICROBIAL METABOLISM AND CARDIOVASCULAR DISEASE

Hazen, S.L.*
Cleveland Clinic, Cleveland, OH USA

Recent studies reveal a novel mechanistic link between intestinal microbiota and the development of cardiovascular disease (CVD). In this presentation, Dr Hazen will show that the gut microbiome can be considered as a large endocrine organ–generating biologically active signaling molecules that influence host phenotypes similar to a hormone. Studies presented will show how dietary nutrients abundant in a Western diet can be acted upon by gut microbes to form metabolites that have biological activity and foster pro-atherosclerotic and thrombotic phenotypes in the host. One example that will be discussed is trimethylamine-N-oxide (TMAO). A gut microbiota dependent metabolite, TMAO in animal model studies has been shown to promote atherosclerosis and thrombosis. And in human clinical studies, TMAO strongly associates with incident risks for MI, stroke or death. Another microbial metabolite recently linked to cardiovascular disease risks, is phenylacetylglutamine (PAG). This metaorganismal (derived from both microbe and host) metabolite is elevated in diabetics, and predicts incident risks for cardiovascular events independent of blood glucose levels. Mechanistic studies reveal PAG serves as a modulator of adrenergic receptor (ADRs) signaling, and mediates cardiovascular relevant phenotypes in part via interactions with ADRs. Moreover, studies suggest some of the beneficial effects of beta blocker therapy may arise from the ability of beta blockers to prevent the adverse effects of elevated PAG levels. Finally, studies presented indicate that the gut microbiome may serve as a potential therapeutic target for the treatment and prevention of CVD and metabolic disease.

References

1. Witkowski M, Weeks TL, Hazen SL. Gut microbiota and cardiovascular disease. Circ Res. 2020;127(4):553-570.
2. Nemet I, Prasad Saha P, Gupta N, Zhu W, Romano KA, Skye SM, Cajka T, Mohan ML, Li L, Wu Y, Funabashi M, Ramer-Tait AE, Naga Prasad SV, Fiehn O, Rey FE, Tang WHW, Fischbach MA, DiDonato JA, Hazen SL. A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors. Cell. 2020;180(5):862-877.
3. Roberts AB, Gu X, Buffa JA, Hurd AG, Wang Z, Zhu W, Gupta N, Skye SM, Cody DB, Levison BS, Barrington WT, Russell MW, Reed JM, Duzan A, Lang JM, Fu X, Li L, Myers AJ, Rachakonda S, DiDonato JA, Brown JM, Gogonea V, Lusis AJ, Garcia-Garcia JC, Hazen SL. Development of a gut microbe-targeted nonlethal therapeutic to inhibit thrombosis potential. Nat Med. 2018;24(9):1407-17.

Friday, July 29 **Gut Microbiota**

0945-1145 Session II: Out of the Box: Impact of Gut Microbes on Non-GI Disease

*—Indicates Presenter

THE MICROBIOME AND CANCER IMMUNOTHERAPY

Shaikh, F.*
John Hopkins University, Baltimore, MD USA

While immune checkpoint inhibitors (ICI) have revolutionized the treatment of cancer by producing durable antitumor responses, only 10%-30% of treated patients respond, and the ability to predict clinical benefit remains elusive. Several studies suggest the gut microbiome may be a modifiable biomarker for tumor response rates, but the specific bacteria or bacterial communities putatively impacting ICI responses have been inconsistent across the studied populations. Further, the mechanisms that drive these interactions are poorly defined in both human and murine models. We have used a combination of approaches to address gaps in the field and further define the mechanisms that drive immunologic interactions. First, we reanalyzed publicly available raw 16S rRNA amplicon and metagenomic sequencing data across five published cohorts (*n*=303 unique patients) using a uniform computational approach to identify novel bacterial signals associated with clinical responders or nonresponders. Further, we developed an integrated microbiome prediction index. Unexpectedly, the NR-associated integrated index shows the strongest and most consistent signa. We subsequently tested the integrated index using validation cohorts across three distinct and diverse cancers (*n*=105). Second, we are now examining some of these key microbes in a human cohort with correlating clinical data. Finally, human gut microbial species found to associate with clinical responses to ICIs are often tested in mice using fecal microbiota transfer (FMT), wherein tumor responses in recipient mice may recapitulate human responses to ICI treatment. Using this model, we define experimental variables that affect anti-tumor responses, taxa that correlate with anti-tumor response, and differential transcriptional changes, with upregulation of T and NK cell-related pathways in mice that received FMT from responder patient. Overall, our human and murine studies identify several species that may play a role in clinical responses to ICIs and suggests attention to biological variables is needed to improve reproducibility and limit variability in murine studies.

SPINGHOLIPIDS AS MODULATORS OF MICROBIOME FUNCTION

Johnson, E.L.*
Cornell University, Ithaca, NY USA

Abstract unavailable at press time.

SPECIFIC GUT BACTERIA REGULATE INNATE IMMUNE FUNCTIONS IN THE BRAIN OF AGED MICE RELATED TO ALZHEIMER'S DISEASE

Wasén, C.;* Cox, L.M.; Vincentini, J.; , J.R.; Lobo, E.L.D.C.; O'Brien, M.; Weiner, H.L.
Ann Romney Center for Neurological Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Introduction. Accumulation of amyloid-β (Aβ) peptides in the brain is an early feature of Alzheimer's disease (AD). Microglia and monocytes clear Aβ, but this function declines in aging and in AD. We have correlated specific gut bacteria to the Aβ levels in AD mice. Bacteria belonging to the *Erysipelotrichaceae* family were associated with low Aβ levels and *Bacteroides* were associated with high levels. In addition, AD mice colonized with *Bacteroides fragilis* had more amyloid plaques (Cox, *et.al.* 2019).

Method. Aged C57BL/6J mice were treated with *Erysipelotrichaceae* (*Faecalibaculum rodentium*, *Ileibacterium valens*, *Dubosiella newyorkensis*) or *B. fragilis* by weekly oral gavages. FITC-conjugated Ab-42 (FITC-Aβ) peptides were injected into the hippocampus and Aβ uptake by microglia and monocytes was assessed by flow cytometry 14-18h later. APP/PS1 transgenic AD mice were treated with *B. fragilis* weekly starting at 2.5 months of age. The mice were sacrificed at 5 months of age, and the microglia transcriptional signature was analyzed.

Results. Female and male WT mice treated with *Erysipelotrichaceae* between 14.5-16.5 months of age had a 2.5-fold higher uptake of FITC-Aβ by microglia compared to mice treated with PBS ($p=0.029$). The Aβ uptake by monocytes was similar in both groups. Male WT mice treated with *B. fragilis* between 8-10 or 12-14 months of age (pooled) had a 1.9-fold reduction in FITC-Aβ uptake by monocytes ($p=0.013$), and a similar trend could be seen in microglia (1.7-fold reduction, $p=0.097$). In APP/PS1 mice, colonization of the gut with *B. fragilis* suppressed expression of 54 genes and increased expression of 10 genes in microglia. Enriched KEGG pathways included lysosome, the phagosome, protein processing in the endoplasmic reticulum, autophagy, and FCgR-mediated phagocytosis. Among down regulated genes were Psen1, Itm2b, and Itm2c that participate in Aβ processing and aggregation, mutations in Psen1 that cause loss of function have been found in familial AD.

Conclusions. Bacteria belonging to the *Erysipelotrichaceae* family stimulated Aβ uptake by microglia, indicating that they may have a protective role against plaque formation in AD. *B. fragilis* inhibited the uptake of Aβ, suppressed cellular pathways involved in the processing, aggregation and degradation of Aβ and may have a detrimental role in amyloid pathology.

References

Cox, L. M., *et.al.* (2019). Scientific Reports 9(1): 17904.

CLOSTRIDIUM PERFRINGENS EPSILON TOXIN AS A POSSIBLE ENVIRONMENTAL CAUSE OF MULTIPLE SCLEROSIS IN HUMANS

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Multiple sclerosis (MS) is an immune-mediated disease of the human central nervous system characterized by blood brain barrier (BBB) permeability and demyelination. MS is believed to occur in genetically predisposed individuals exposed to an unknown environmental stimulant. Here, we present clinical and experimental data that indicates that *Clostridium perfringens* (*C. perfringens*) epsilon toxin (ETX) may be a causative agent of MS. Clinically, we demonstrate that MS patients, compared to healthy controls, have increased exposure to ETX as well as the *C. perfringens* types B and D strains that produce it. Using novel culture techniques to detect low abundance *C. perfringens* strains in human fecal samples, we find that 29% of MS patients versus 3.2% of healthy controls are colonized with ETX producing *C. perfringens* strains. Importantly, we successfully isolated and sequenced a MS-derived *C. perfringens* type D strain. Experimentally, we demonstrate that ETX recapitulates MS related pathology including BBB permeability and demyelination in several mouse models. Taken together, this data strongly supports the hypothesis that ETX may be an environmental trigger for initiating MS in humans.

July 28-31

Friday, July 29 — Fusobacteria

1330-1515 Session III: Fusobacteria

*—Indicates Presenter

MECHANISTIC INSIGHT OF *FUSOBACTERIUM NUCLEATUM* IN COLORECTAL CANCER

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Fusobacterium nucleatum is a gram-negative anaerobe ubiquitous in the oral cavity. It plays a critical role in dental plaque formation and is prevalent in periodontal disease. It is also one of the most prevalent species involved in extra-oral infections and inflammation, including a broad spectrum of cancers. *Fn* is the most enriched species in the fecal microbiome of colorectal cancer patients suggesting oral-GI translocation. FadA adhesin is a key virulence factor uniquely encoded in *Fn* required for the bacterium to bind and invade host cells. Although FadA is constitutively expressed, it undergoes dramatic biochemical change under stress and disease conditions to turn into an amyloid-like isoform, which is secreted via Type V autotransporter Fap2. Amyloid FadA mediates biofilm formation, confers acid tolerance, binds cancer cells and upregulates Annexin A1 expression to activate ß-catenin signaling and promote cancer cell growth. Our study elucidates a novel paradigm for a benign commensal to turn into a virulent pathogen.

EXPERIMENTAL APPROACHES TO IDENTIFY VIRULENCE DETERMINANTS IN *FUSOBACTERIUM NUCLEATUM*

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A Gram-negative colonizer of the oral cavity, *Fusobacterium nucleatum* interacts with many microbes in the oral microbiome, facilitating development of oral biofilms. This anaerobe can also spread to extraoral sites including placenta and colon, promoting preterm birth and colorectal cancer. To date, however, the molecular mechanisms of interspecies interactions, or coaggregation, biofilm formation, and fusobacterial virulence are not well understood. Here, we employed a combination of reverse and forward genetics, biochemical methods, and *in vivo* models of infection to reveal a new set of factors that contribute to the virulence potentials of *F. nucleat*

FUSOBACTERIA-DOMINANT COLORECTAL CANCER BIOFILMS ARE ASSOCIATED WITH HIGH LEVELS OF THE POLYAMINE N1,N12-DIACETYLSPERMINE

Drewes, J.L.;*[1] Cai, Y.;[2] Wensel, C.R.;[1] Queen, J.;[1] Wanyiri, J.;[1] Roslani, A.C.;[3] Vadivelu, J.;[3] Johnson, C.H.;[2] Sears, C.L.[1]
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Mucus-invasive colonic biofilms (BFs) are dense, polymicrobial communities that are prevalent in colorectal cancer (CRC), are pro-tumorigenic in mouse models, and are associated with elevated levels of the polyamine N1,N12-diactylspermine (DAS). Polyamines such as DAS, which can be produced by either the host or the gut microbiota, have been associated with host cell proliferation including in colorectal cancer (CRC) and are required for the growth and biofilm formation of at least some bacteria. However, whether BF composition impacts DAS levels and/or vice versa is presently unknown. To better understand this relationship, Carnoy's-fixed CRC resections from 115 patients from the Universiti Malaya in Malaysia were screened for mucus-invasive BFs with probes for all bacteria (EUB338), Bacteroidetes (Bac), Fusobacteria (Fuso), Lachnospiraceae (Lac), and β/γ-Proteobacteria (Proteo), and imaged on a Zeiss780 confocal microscope. Untargeted metabolomics was performed on 5-10 samples of each BF type using a Waters UPLC Xevo G2-XS quadrupole time-of-flight (QTOF) mass spectrometer. Seventy-nine of 115 Malaysian tumors (69%) were BF+ using EUB338 staining. Of the BF+T, 55% were polymicrobial (predominantly Bac/Lac), 40% were polymicrobial with Fuso, and 5% were Proteo dominant. Untargeted metabolomics of BF- vs. BF+ tumors with various BF composition subtypes revealed a gradient in levels of the polyamine DAS, with BF+T with Fuso blooms having the highest levels ($p = 0.055$ vs. Proteo-dominant BF+T, $p = 0.043$ vs. Bac/Lac BF+T), followed by Proteo-dominant and Bac/Lac BF+T, BF-T, and finally BF- healthy biopsies. These data suggest that Fuso-dominant BFs may drive DAS production in CRC tissues or, alternatively, that colonic tissue sites with high levels of DAS are important niches for Fuso-dominant BFs.

FUSOBACTERIUM NUCLEATUM SUBSPECIES DIFFER IN BIOFILM FORMING ABILITY *IN VITRO*

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Emergence of dysbiosis in bacterial biofilms that form on teeth triggers periodontitis, a major cause of tooth loss. *Fusobacterium nucleatum*, a key organism in the formation of subgingival biofilms, bridging early colonisers to periodontal pathogens that drive such dysbiosis, is associated with periodontitis, and also with systemic conditions, such as colorectal cancer. Five subspecies of *F. nucleatum* have been identified: *animalis, fusiforme, nucleatum, polymorphum* and *vincentii*. Differences in the integration of subspecies into multispecies biofilm models have been reported. The purpose of this study was to examine single subspecies *F. nucleatum* biofilm formation, hitherto un-studied.

Static single subspecies *F. nucleatum* biofilms were grown anaerobically for three days on untreated or surface-modified (sandblasting or fibronectin, gelatin or poly-L-lysine coating) plastic and glass coverslips. Biofilm mass was quantified using crystal violet (CV) staining. Biofilm structure and thickness were analysed by scanning electron microscopy and confocal laser scanning microscopy.

Surface type and treatment had differential effects on single-subspecies biofilm formation. Biofilm formation was overall highest on poly-L-lysine coated surfaces and sandblasted glass surfaces. *F. nucleatum*, ssp. *polymorphum* did not form a stable layer detectable by CV on any of the tested substrates.

Here, we show that biofilm formation by *F. nucleatum in vitro* is subspecies- and substrate-specific. Furthermore, not all subspecies appear to be able to form stable single subspecies biofilms in vitro as shown by *F. nucleatum,* ssp. *polymorphum*. Future studies will investigate the molecular mechanisms of *F. nucleatum* subspecies adhesion and biofilm formation to shed light on subspecies-specific differences reported in this study. Understanding these mechanisms may subsequently reveal new virulence factors as novel therapeutic targets for prevention and treatment of *F. nucleatum*-mediated diseases.

July 28-31

Friday, July 29 — Methodology

1330-1515 Session IV: Mothodology and Epidemiology

⁎—Indicates Presenter

UNDERSTANDING THE ROLES OF TCDE AND TCDL IN TOXIN SECRETION

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Understanding how large macromolecules are transported across a cell wall is a complex and poorly understood biological process. The nosocomial pathogen *Clostridioides difficile* produces two large toxins, TcdA (308 kDa) and TcdB (270 kDa). Although toxin function in host cells has been extensively studied, little is known about how these toxins are secreted from the bacterium. TcdA and TcdB are encoded on a pathogenicity locus (*PaLoc*), which also encodes the holin-like protein TcdE and the remnants of a partial endolysin TcdL. While bacteriophages use holin/endolysin systems to trigger bacterial cell lysis and escape, multiple reports now suggest that TcdE is used for the secretion of the toxins by forming a channel within the cell membrane. While TcdL was only recently discovered, there are data to suggest that it can interact with TcdB to help facilitate toxin translocation. To further understand toxin secretion, we created strains with *tcdE*, *tcdL*, or *tcdE* and *tcdL* deleted. Our data indicate that both TcdE and TcdL are required for secretion. Furthermore, we found that TcdA and TcdB are secreted in a temporal manner where TcdA is secreted before TcdB. The research presented here will address the outstanding questions of how TcdE and TcdL interact with *C. difficile* toxins to create a pore and how the toxins can be released with mechanisms other than cell lysis.

IDENTIFICATION OF A BILE ACID-BINDING TRANSCRIPTION REGULATOR IN *CLOSTRIDIOIDES DIFFICILE* USING CHEMICAL PROTEOMICS

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Clostridioides difficile is a Gram-positive anerobic bacterium that is the leading cause of hospital-acquired gastroenteritis in the US. *C. difficile* experiences many insults during its traverse through the human gut, including bile acids that inhibit its growth. *C. difficile*'s sensitivity to toxic bile acids has been hypothesized to limit *C. difficile* infection because the levels of certain bile acids correlate with susceptibility to infection. Since many gut bacteria encode mechanisms to sense and adapt to toxic bile acids, we sought to uncover mechanisms by which *C. difficile* adapts to bile acids using a chemical proteomics screen. Our photoactivatable bile acid probes identified an uncharacterized MerR-family protein, CD3583, as a putative bile acid-sensing transcription regulator. We have shown that CD3583 binds and appears to be stabilized by lithocholic acid (LCA) in *C. difficile*. Interestingly, CD3583's affinity for deoxycholic and chenodeoxycholic acids (DCA and CDCA, respectively) is reduced, and this difference in affinity correlates with their toxicity to *C. difficile* in vitro. Although deletion of cd3583 did not affect *C. difficile*'s sensitivity to LCA, it caused cells to elongate more in the presence of LCA. Since cell elongation in response to LCA has been previously reported, our data suggest a possible role for CD3583 in control of cell length. We are currently investigating transcriptional responses of *C. difficile* to LCA, including its dependency on CD3583. Future studies will include analysis of CD3583's ability to bind DNA and regulate gene expression, as well as the role of CD3583-dependently expressed genes in *C. difficile*'s ability to tolerate LCA. The *C. difficile* toxin TcdB has also been shown to bind bile acids, and as such, our identification of a bile acid-binding transcription factor highlights the importance of these molecules in regulating *C. difficile* infection.

NATURAL MUTATION IDENTIFIED IN CDTR OF CLINICAL *CLOSTRIDIOIDES DIFFICILE* ISOLATE ATTENUATES VIRULENCE IN MICE

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Clostridioides difficile (*C. difficile*) strains belonging to the epidemic BI/NAP/027 group have been associated with increased transmissibility and disease severities. In addition to the major virulence factors toxin A and toxin B, RT027 strains also encode binary toxins cdtA and cdtB. However, the impact of binary toxins on clinical severity is controversial. Our lab has examined virulence of clinical strains using mouse models and identified two RT027 strains that do not cause diarrhea or morbidity in antibiotic-treated mice, despite high colonic colonization and comparable toxin expression in feces. Genomic comparison of virulent and avirulent RT027 strains identified a 69 base-pair (23 amino acids in-frame) deletion in the N-terminus of the cdtR gene, which encodes a response regulator for binary toxin expression. The avirulent RT027 strain does not cause disease in innate immune deficient ($Myd88^{-/-}$) or germ-free mice, suggesting that neither host immunity nor microbiome-mediated colonization resistance are responsible for reduced *in vivo* pathogenesis. Lower levels of toxin transcription were detected in cecal contents but not fecal pellets when germ-free mice were infected with the avirulent versus virulent RT027 strains. We used a CRISPR knock-out strategy to generate a cdtR Δ69 deletion mutant in R20291 and demonstrate in the mouse infection model that cdtR is essential for *C. difficile* pathogenicity and that the 23 amino acid deletion ablates cdtR function and *C. difficile* virulence. We are currently investigating the mechanism by which this mutation-mediated loss of function in cdtR markedly reduces *in vivo* pathogenesis. In summary, we describe two distinct clinical strains with a natural mutation in the cdtR gene that leads to reduced toxins production and greatly attenuated virulence in mice. These results demonstrate the important role of cdtR during infection in mice and potentially for increased virulence in RT027 infected patients.

FLIW AND CSRA GOVERN FLAGELLIN (FLIC) SYNTHESIS AND PLAY PLEIOTROPIC ROLES IN VIRULENCE AND PHYSIOLOGY OF *CLOSTRIDIOIDES DIFFICILE* R20291

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Clostridioides difficile flagellin FliC is associated with toxin gene expression, bacterial colonization and virulence, and is also involved in pleiotropic gene regulation during *in vivo* infection. However, how *fliC* expression is regulated in *C. difficile* remains unclear. In *Bacillus subtilis*, flagellin homeostasis and motility are coregulated by flagellar assembly factor FliW, Flagellin Hag (FliC homolog), and CsrA (Carbon storage regulator A), which is referred to as partner-switching mechanism "FliW-CsrA-Hag". In this study, we characterized FliW and CsrA functions by deleting or overexpressing *fliW*, *csrA*, and *fliW-csrA* in *C. difficile* R20291. We showed that *fliW* deletion, *csrA* overexpression in R20291, and *csrA* complementation in R20291ΔWA (*fliW-csrA* codeletion mutant) dramatically decreased FliC production, but not *fliC* gene transcription. Suppression of *fliC* translation by *csrA* overexpression can be relieved mostly when *fliW* was coexpressed, and no significant difference in FliC production was detected when only *fliW* was complemented in R20291ΔWA. Further, loss of *fliW* led to increased biofilm formation, cell adhesion, toxin production, and pathogenicity in a mouse model of *C. difficile* infection (CDI), while *fliW-csrA* codeletion decreased toxin production and mortality *in vivo*. Our data suggest that CsrA negatively modulates *fliC* expression and FliW indirectly affects *fliC* expression through inhibition of CsrA post-transcriptional regulation. In light of "FliW-CsrA-Hag" switch coregulation mechanism reported in *B. subtilis*, our data also suggest that "FliW-CsrA-*fliC*/FliC" can regulate many facets of *C. difficile* R20291 pathogenicity. These findings further aid us in understanding the virulence regulation in *C. difficile*.

COMPARATIVE GENOMIC ANALYSIS OF *CLOSTRIDIOIDES DIFFICILE* STRAIN RESTRICTION ENDONUCLEASE ANALYSIS GROUP Y ACROSS FOUR DECADES

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Despite marked changes in the molecular epidemiology of *Clostridioides difficile* infection (CDI) over the past 40 years, restriction endonuclease analysis (REA) group Y (RT014/020) has always been present. We conducted a comparative genomic analysis of REA group Y isolates in comparison to the completed CD genomes deposited to RefSeq.

Whole genome sequencing (WGS) was performed on 40 isolates previously identified as REA group Y collected from 1985–2015. Comparative genomic analysis between isolates identified as REA group Y and 95 of the completed RefSeq CD genomes was performed by identifying the core genome and comparing the remaining accessory genomic elements (AGE) between Y and non-Y groups.

Among the 135 genomic sequences analyzed, 3,262 AGEs (≥200bp) were identified. The AGEs of REA group Y isolates had a Bray-Curtis dissimilarity of 0.1 on average, indicating a high degree of relatedness. A strong association was found with REA Y sequences and a 10kb coding DNA sequence (CDS) that encodes the principal components of the S-layer cassette genes, *slpA, secA,* and *cwp66* (Cramér's $V = 0.73$, $p < 0.0001$). Among our REA group Y cohort, we found a moderately strong association with an additional *slpA* CDS within the most common REA subgroups, Y1 and Y4, when compared to other REA group Y isolates (Cramér's $V = 0.53$, $p < 0.01$).

REA group Y isolates were found to have a strong association with an S-layer cassette. The S-layer cassette has been shown to play a critical role in epithelial cell adhesion, which is a first critical step for *C. difficile* intestinal colonization. Our findings would indicate that epithelial cell adhesion might differ within REA group Y isolates, providing a competitive advantage for colonization. Moreover, REA subgroups Y1 and Y4 appear to encode a distinct *slpA*, which could further explain the long-term persistence of these 2 subgroups. It is not yet known if these AGEs have a different phenotypic expression, which might confer a competitive advantage regarding epithelial cell adhesion and colonization.

ANAEROBIC MICROBIOTA FACILITATE PATHOGEN ACCESS TO THE AIRWAY EPITHELIUM IN A NOVEL CO-CULTURE MODEL OF COLONIZATION

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Culture-independent analyses of human microbiota have implicated strict and facultative anaerobic bacteria in the onset and progression of chronic bacterial airway disease. However, the mechanistic bases of their contributions to disease pathophysiology are poorly understood due to the inherent limitations of existing laboratory models and conflicting oxygen demands between anaerobes and host cells. To overcome these limitations, we developed and optimized a daul oxic/anoxic co-culture approach that maintains an oxygen-limited microenvironment at the epithelial interface while host cells are oxygenated basolaterally. We first demonstrated that reduced oxygen culture conditions did not compromise the viability, tight junction integrity, or inflammatory response of airway epithelial cells, while RNAseq revealed few changes in their transcriptomic profile. Using individual anaerobic bacteria *(*e.g. *Fusobacterium nucleatum, Prevotella melaninogenica, Streptococcus* parasanguinsis) and a mixed anaerobic bacterial community derived from the airways of a cystic fibrosis patient, we confirmed that our co-culture model can sustain host-anaerobe interactions for ~72 h without affecting the viability of both host and anaerobic bacterial cells. We show that in response to anaerobe challenge, apical mucin integrity is significantly altered as is the expression of a panel of inflammatory marker genes. Single-cell RNA sequencing also revealed the tropic response of airway epithelial cells to anaerobe challenge. Preliminary observations led to our hypothesis that anaerobe-mediated mucus degradation primes the airway epithelium for colonization by canonical pathogens. Indeed, treatment of a mucus-overproducting cell line (Calu-3) with an anaerobic bacterial consortium prior to challenge with *Pseudomonas aeruginosa* and *Staphylococcus aureus* led to a significant increase in pathogen colonization. Altogether, this novel anaerobic co-culture model system offers new insight into anaerobe-host interactions and the potential role of anaerobic microbiota in the onset and progression of chronic airway disease.

July 28-31

Friday, July 29 **Bugs as Drugs**

1530-1730 Session V: Bugs as Drugs: Engineered Microbial Communities & Fecal Microbiota Transplant

*—Indicates Presenter

CURRENT STATE OF KNOWLEDGE: FECAL MICROBIOTA TRANSPLANT FOR FECAL MDRO COLONIZATION

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This presentation will review the current state of knowledge of the role of the fecal microbiome as a reservoir for multidrug-resistant organism (MDRO), and the role of fecal microbiota transplantation (FMT) to prevent or reverse fecal MDRO colonization. We will discuss the concept of colonization resistance and the factors that influence the gut microbiome, review the role of FMT in medicine and the impact of FMT on the gut microbiome. We will discuss the practical and research needs necessary to understand the role of FMT for fecal MDRO colonization, and future directions.

CONSISTENT MICROBIOME AND BILE ACID RESTORATION ACROSS THREE CLINICAL TRIALS OF RBX2660 FOR RECURRENT *CLOSTRIDIODES DIFFICILE* INFECTION: A COMBINED ANALYSIS

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PURPOSE: To evaluate the changes in microbiome and bile acids signatures across multiple studies of RBX2660, an investigational microbiome-based live biotherapeutic.

BACKGROUND: Recurrent *Clostridioides difficile* infections (rCDI) are an urgent public health threat associated with disruption of the microbiome. RBX2660, a microbiota-based investigational live biotherapeutic, has been evaluated in >600 clinical trial participants for reducing recurrence of rCDI. Here we report the combined microbiome and metabolomic analysis of participants in three trials of RBX2660.

METHODS: Samples from RBX2660- or placebo-treated participants from the PUNCH CD2 (n=153), PUNCH CD3 (n=887) and PUNCH OLS (n=653) studies were included. Clinical response was defined as the absence of CDI recurrence at eight weeks post-treatment. Volunteer stool samples were collected prior to blinded treatment (BL), 1, 4 and 8 weeks, up to 24 months post-treatment. Samples were sequenced using shallow shotgun methods. Operational taxonomic unit (OTU) data were used to calculate relative taxonomic abundance, alpha diversity, and the Microbiome Health Index™ (MHI-A)—a microbiome biomarker of post-antibiotic dysbiosis and restoration. Liquid chromatography mass spectrometry was used to quantify 33 bile acids for participant samples from PUNCH CD2 and CD3.

CONCLUSION: Relative to BL, microbiome diversity and composition shifted significantly in treatment responders for all trials, with greater shifts documented among RBX2660-treated responders. Bacteroidia and Clostridia increased and Gammaproteobacteria and Bacilli decreased after treatment. MHI-A was restored from dysbiotic to healthy levels (less so among placebo responders). In PUNCH CD2 and CD3, bile acid compositions were restored to a composition more resistant to *C. difficile* infection. Collectively, RBX2660 restored microbiome and bile acid compositions concurrent with clinical response. Restorative changes are characteristic of shifts from a post-antibiotic dysbiosis to a healthier state.

IMPACT OF SER-109, AN INVESTIGATIONAL -MICROBIOME THERAPEUTIC, ON STOOL FATTY ACID AND BILE ACID METABOLITES IN A PHASE 3 RANDOMIZED TRIAL (ECOSPOR III) FOR TREATMENT OF RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION (rCDI)

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In a Phase 3 double-blind, randomized trial, SER-109, an oral investigational microbiome therapeutic composed of purified Firmicutes spores, was superior to placebo in reducing risk of rCDI at week 8, the primary endpoint. Compared to placebo, SER-109 led to higher engraftment of dose species at weeks 1, 2 and 8. Here, we evaluated changes in microbially mediated short, medium and branched-chain fatty acids, as well as bile acid profiles (primary vs secondary), based on the hypothesized role of these metabolites in inhibiting spore germination and replication of toxin-producing *C. difficile.*

Concentrations of fatty acids, as well as primary and secondary bile acids, in subjects' stool samples were measured from pretreatment ("baseline") to 1, 2, and 8 weeks by targeted liquid chromatography with tandem mass spectrometry. Two-sided Mann-Whitney U tests were used to determine statistical significance.

Baseline concentrations of stool metabolites were comparable between the two arms. At week 1, concentrations of metabolites thought to inhibit *C. difficile* replication (short-chain fatty acid butyrate, medium-chain fatty acids valerate and hexanoate, and secondary bile acids) rapidly and significantly increased and remained significantly higher at week 2 ($p < 0.01$, Mann-Whitney U test) and week 8 ($p < 0.05$, Mann-Whitney U test) in the SER-109 arm compared to placebo. Conversely, concentrations of primary bile acids, known germinants of *C. difficile* spores, were significantly reduced at week 1 ($p < 0.05$, Mann-Whitney U test) in the SER-109 arm compared to placebo. These changes correlated with a greater magnitude of engraftment of SER-109 dose species compared to placebo through week 8 and were associated with a reduction in rCDI events. These data suggest that some mechanisms of action of improved clinical outcomes may be through increasing fatty acid production, along with rapid conversion of primary to secondary bile acids, thereby interrupting the two-phase life cycle of *C. difficile*.

EFFICACY AND SAFETY OF SER-109, AN INVESTIGATIONAL MICROBIOME THERAPEUTIC FOR TREATMENT OF RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION (rCDI): A PHASE 3 DOUBLE-BLIND, RANDOMIZED TRIAL (ECOSPOR III)

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Antibiotics alone are often insufficient to treat *C. difficile* infection (CDI) because they have no effect on *C. difficile* spores that germinate within a disrupted microbiome. SER-109, an investigational, oral microbiome therapeutic comprised of purified Firmicutes spores, was designed to reduce rCDI through microbiome repair. Here, we report efficacy and safety data through week 24 from ECOSPOR III, a double-blind, placebo-controlled trial of SER-109.

Adults with rCDI (≥3 episodes in 12 months) were screened at 56 US/Canadian sites. After completing standard-of-care antibiotics, subjects were randomized 1:1 to SER-109 (4 oral capsules x 3 days) or placebo. The primary endpoint was rCDI (recurrent toxin+ diarrhea requiring treatment) through week 8; secondary endpoints included efficacy and safety through 24 weeks.

281 subjects were screened and 182 randomized (59.9% female; mean age 65.5 years). SER-109 was superior to placebo in reducing rCDI at week 8 (12.4% vs 39.8%, respectively); relative risk (RR), 0.32 [95% CI, 0.18-0.58; P<0.001 for RR<1.0; P<0.001 for RR<0.833], consistent with a relative risk reduction of 68%. Significantly lower rates of rCDI in SER-109-treated subjects compared to placebo were observed as early as 4 weeks posttreatment and were maintained through 24 weeks (absolute risk reduction of 22.1%, 27.4%, 28.3%, and 26.0% at weeks 4, 8, 12, and 24, respectively). SER109 was well-tolerated with a safety profile similar to placebo. Mild to moderate gastrointestinal symptoms were the most common treatment-emergent adverse events (TEAEs). No serious TEAEs or deaths were deemed related to treatment.

SER-109 was superior to placebo in reducing rCDI at week 8, with a safety profile comparable to placebo. The favorable impact of SER-109 was observed as early as week 4 and sustained through 24 weeks, highlighting the clinical benefit of early microbiome repair in this two-pronged therapeutic approach.

July 28-31

Friday, July 29 — Oral Microbiome

1530-1730 Session VI: The Oral Microbiome and Human Health

*—Indicates Presenter

PRECISION-GUIDED ANTIMICROBIAL THERAPY FOR TARGETED MODULATION OF HUMAN MICROBIOME

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One major challenge to studying human microbiome and its associated diseases is the lack of effective tools to achieve targeted modulation of individual species and study its ecological function within multispecies communities. We will report various approaches to achieve precision-guided antimicrobial therapies in modulating oral microbiome, including a specifically targeted antimicrobial peptide and a pH sensitive molecule, respectively. These engineered molecules showed their selective killing abilities within complex oral microbial communities with high efficacy. Most interestingly, targeted removal of specific pathogens allow significant shift in the overall microbial structure, leading more healthy microbial communities, demonstrating these approaches are powerful tools to identify and characterize “key pathogens” within microbiome.

ANAEROBIC *PAS DE DEUX:* INTRODUCING *PARVIMONAS MICRA* GENETICS AND ITS INTERSPECIES AND HOST INTERACTIONS

Merritt, J.L.*
Oregon Health & Science University, Portland, OR USA

Parvimonas micra is a member of the largely uncharacterized Tissierellia class of the Firmicutes and a common constituent of the microbiomes of multiple mucosal sites in the human body. *P. micra* is widely recognized as one of the most common sources of clinical infections caused by Gram-positive anaerobic cocci (GPAC), where it typically serves as a major pathobiont in a wide range of mucosal dysbiotic diseases and various types of malignant tumors. Despite the wealth of evidence supporting its role in various diseases, almost nothing is known about *P. micra* genetics or its pathobiology, largely as a consequence of its fastidious nature and genetic intractability.

In an attempt to better understand its role in human disease, we isolated a collection of *P. micra* strains directly from human odontogenic abscess clinical specimens and then screened the isolates for natural competence. We were surprised to discover that natural competence is quite common among both *P. micra* isolates and established laboratory strains, making it the only known naturally competent species within the entire Tissierellia branch of the Firmicutes. We exploited *P. micra* natural competence to create the first tractable genetic system for this species. In addition, we have also investigated *P. micra* interactions with other bacteria and host cells. We observed robust dual species *P. micra-F. nucleatum* biofilm formation, with *F. nucleatum* exhibiting intriguing morphological phenotypes in the presence *P. micra. P. micra* also appears to modulate neutrophil behaviors, firstly by stimulating unusually aggressive phagocytosis and then by preventing their subsequent destruction within phagosomes. Surprisingly, both *P. micra* and their host neutrophils appear to be viable and healthy following phagocytosis. The implications of these studies will be discussed, especially as they relate to synergistic polymicrobial infections.

EXPERIMENTAL GINGIVITIS: LESSONS LEARNED FROM CHEMOKINE MULTI-PLEX AND BACTERIAL SEQUENCE ANALYSIS

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In this presentation the results from two human studies: one, determining the variability of the innate host response in healthy individuals, and two characterizing the variability in the innate host response and microbial composition changes during experimental gingivitis. It was found that in health, there are distinct variations within individual gingival crevicular fluid chemokine expression profiles, as well as in the concentration of neutrophils, that divided the participants into high or low chemokine expressing groups. Furthermore, species characterization of healthy subgingival plaque revealed significant inter-individual variability that identified two unique groups unrelated to the previously identified chemokine groups. The lack of concordance between the microbial composition and chemokine profile during health may be due to the fact that this brief snap-shot in time may have only captured a transient moment within the larger regulatory context and may not affect overall health or disease. This would suggest that the dynamics normally associated with periodontal health fluctuate within healthy individuals. For example, this study did not consider variations within individual diets, nor account for differences within natural circadian rhythms. In the second human experimental gingivitis study three unique clinical inflammatory phenotypes (high, low, and slow) were identified. It was found that in the slow response group, IL-1β, a reported major gingivitis associated inflammatory mediator, was not associated with clinical gingival inflammation and in addition, significantly higher levels of *Streptococcus* spp. were also unique to this group. The low clinical response group was characterized by low concentrations of host mediators, despite similar bacterial accumulation and compositional characteristics as the high clinical response group. Neutrophil and bone activation modulators were downregulated in all response groups, revealing novel tissue and bone protective responses during gingival inflammation. These alterations in chemokine and microbial composition responses during experimental gingivitis reveal a previously uncharacterized variation in the human host response to a disruption in gingival homeostasis.

A GENOMIC PORTRAIT REVEALS THE GENETIC PLASTICITY AND EVOLUTIONARY ADAPTATION OF *PORPHYROMONAS GINGIVALIS*

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Porphyromonas gingivalis is a Gram-negative oral anaerobe that is involved in the pathogenesis of periodontitis. However, the lack of worldwide genomics-based studies of *P. gingivalis* hampers understanding the evolutionary pathways underlying its virulence and adaptation to humans.

We aimed to elucidate the population structure of *P. gingivalis* and to determine the evolutionary mechanisms underlying its virulence and adaptation. Hence, we applied the pan-genome approach on 77 publicly available *P. gingivalis* genomes (most were from Japan (n=42), the Netherlands (n= 10), the USA (n=3), and Sudan (n=3)). In addition, all genomes were screened for the presence/absence of antimicrobial-resistant and virulence genes.

The pan-genome *P. gingivalis* was made up of 5831 genes with 63% of them classified as cloud genes (1% <= strains < 15%), while the core genes (95% <= strains <= 100%) constituted only 24% and the remaining 13% assigned as shell genes (15% <= strains < 95%), together suggesting a high intraspecies diversity. In addition, 88% of the *P. gingivalis* genomes had a novel multilocus sequence type (MLST). Two major phylogenetically distinct clades became apparent: clade 1 genomes harbored genes involved in e.g. galactose catabolism and bacteriocin production (DfsB protein, competition among sibling bacterial colonies), while clade 2 genomes were characterized by carrying CRISPR-Cas and glycosyltransferase genes. Only two genomes harbored genes encoding resistance to macrolide and tetracycline antibiotics, overall, not suggesting the emergence of antibiotic resistance. Interestingly, all 77 genomes harbored the *pgpB* gene, which is responsible for polymyxin B (a cationic polypeptide antibiotic) resistance and for evading Toll-like receptor 4 (TLR4)-mediated innate immune response. In conclusion, our results show that *P. gingivalis* has evolved on several occasions to defend itself against other microorganisms including phages and to succeed in the killing battle with the human innate immune response.

A REVISED TAXONOMY OF THE GENUS *TREPONEMA* DELINEATING COMMENSAL AND PATHOGENIC ORGANISMS

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Shifts in human subsistence lifestyles, specifically the adoption of industrial agricultural practices has resulted in the reduced prevalence and even complete absence of certain microbial taxa in the gut microbiota of industrialized populations. Among these "missing microbes," members of the genus *Treponema* have been of particular interest, with numerous studies documenting their prevalence and diversity in the gut microbiome of various extant human populations. While challenges with sample preservation and transport have limited the recovery of gut *Treponema* from non-industrial human populations, recent advances in metagenome sequencing and analysis have helped determine the phylogenetic and functional diversity of these organisms. In our continuing studies of hunter-gatherer and rural-agriculturalist populations, analysis of shotgun metagenome datasets recovered 25 high-quality metagenome-assembled genomes (MAGs, >90% completion), grouped into four species clusters. Phylogenomic comparison of the *Treponema* MAGs with other validly published *Treponema* genomes showed that human gut *Treponema* were most closely related to the organisms isolated from bovine and porcine gut ecologies. Furthermore, these commensal gut-associated organisms formed clades that were distinct from pathogenic *Treponema* including *T. pallidum*, and *T. denticola*. A comprehensive analysis integrating phylogenomics, genome-wide identity scores (AAI, ANI), and *in silico* screening for functional genes (including those associated with carbohydrate metabolism), showed that the genus *Treponema* should be restructured into 14 novel genera using current taxonomic standards. This taxonomic revision distinguishing the commensal gut clades from the more commonly recognized pathogenic treponemes is essential as we explore their role and acceptance as potential therapeutics to restore healthy gut function.

Saturday, July 30 **Gastrointestinal Tract**

0800-0945 Session VII: Functional Roles of Anaerobes in the Gastrointestinal Tract

*—Indicates Presenter

GLYCAN SCAVENGING BY HUMAN GUT BACTERIA

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Competition for dietary carbohydrates influences the development and stability of the mammalian gut microbiota. An understanding of the molecular details that drive the ability of bacteria to utilize dietary carbohydrates may allow the design of prebiotic and probiotic therapies to feed select species for desired health outcomes. Starch is an abundant dietary fiber in the Western diet, and the form that typically transits the distal gut, termed resistant starch, has a profound influence on the structure and metabolic output of the gut community. *Ruminococcus bromii* is a Gram-positive anaerobe with the rare ability to degrade insoluble granules of dietary starch that escape digestion by host enzymes in the upper gastrointestinal tract. To bind and degrade starch, *R. bromii* synthesizes amylosomes, complexes of starch-binding proteins and starch-hydrolyzing enzymes that are tethered to the cell surface. The goal of our work is to understand the molecular features of the starch-targeting proteins unique to the amylosome and not found in organisms that utilize soluble starch. We have recently determined the crystal structures of the amylosome proteins Sas6 and Sas20, two non-catalytic proteins that adhere to granular corn starch. Sas6 features a CBM74 domain that binds both single- and double-helical stretches of amylose and amylopectin, the two main components of starch granules. The a-glucan chain is bound along an extended central cavity via hydrogen-bonding rather than hydrophobic interactions. Sas20 features two starch-binding domains, including one that allows the protein to dock to the amylose and amylopectin non-reducing ends that are enriched at the starch granule surface. An interesting feature of Sas20 is its flexible conformation in solution, which may enhance its ability to find and capture these polysaccharide chain ends. Both proteins are tethered to the cell-surface via calcium-dependent protein-protein interactions, and future work is directed at understanding the assembly of this complex. Our work not only demonstrates how a dedicated starch-degrading organism recognizes key features of its target polysaccharide, but also reveals novel features of starch-binding domains that could be engineered onto commercial amylases for enhanced hydrolytic performance.

MICROBIAL BILE ACID CONJUGATION AS A NEW MECHANISM OF HOST-MICROBIOME CROSSTALK

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Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI USA

Through 170 years of bile acid chemistry research, comprising over 40,000 publications, our knowledge of mammalian bile acid conjugation was limited to the amino acids glycine and taurine. By screening organs of germ free (GF) and colonized mice with LC-MS/MS based metabolomics, we have discovered unique bile acids made by the microbiome that are produced by acyl-conjugation of cholic acid with alternative amino acids in the gut. These microbially conjugated bile acids (MCBAs) are produced by a multitude of gut anaerobes, and we have been characterizing the breadth of their amino acid conjugation and bile acid substrate specificity. They are prevalent in humans, particularly those with Crone's disease and in infants early in life, but their role in mammalian GI health is poorly understood due to their novelty. MCBA abundance is approximately 10-50-fold lower than the host conjugated taurocholic acid in the murine gut, but there is wide variation in the specific amino acids conjugated in mice. Furthermore, certain MCBAs modify bile acid signaling compared to conjugated bile acids or cholate itself and this depends on the amino acid conjugated. Leucine conjugated cholic acid reduces signaling of the bile acid receptor TGR5 by half, supporting the potential for MCBAs to act as signaling molecules in mammals. We have leveraged a novel knockout mouse model that is devoid of host-conjugated bile acids to better understand how gut anaerobe bile acid conjugation alters mammalian GI metabolism. Preliminary data from the mouse model shows that bile acid conjugation is important for early life development in mammals and MCBAs contribute to this. These experiments have also shown that mammalian bile acid conjugation by the liver is itself poorly understood, as the knockout mice had a uniquely shifted bile acid pool containing even more unique conjugations. Although many medical textbooks describe the role of bile acids and bile acid conjugation in GI health, it is clear from recent research, that we poorly understand how our own enzymes and that from our microbiota contribute to bile acid metabolism.

CHARACTERIZATION OF RARE *LACHNOSPIRACEAE*

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Sequencing-based methods have greatly increased the ability to infer phylogeny or functions beyond widely used 16S rRNA gene-based methods. However, much of what we know about the human microbiota is still limited to taxonomic identification of 'who is there,' which does not provide strain-level resolution or provide information at the functional level. With the exception of species that have been developed into model commensal organisms or demonstrated to be of direct clinical relevance, many indigenous species in the gut microbiota remain highly uncharacterized. This is particularly true for many genera or species belonging to the Clostridia class, which are frequently associated with health or essential functions. To investigate diversity and functional differences of individual bacterial species in the gut microbiota, we developed a cultivation-based pipeline to recover individual strains of Clostridia and Erysipelotrichia in the gut. Most species were classified as *Lachnospiraceae*, which represent a prevalent family within the human fecal microbiota. Phylogenomic and pangenomic analysis of select *Lachnospiraceae* species from our study and publicly available genome datasets suggests diversification within species both within and across hosts. We also observed an abundance of diverse carbohydrate-active enzymes (CAZyme) gene families across *Lachnospiraceae* species, both at the inter- and intra-species levels. Understanding strain diversity in these undercharacterized gut microbes can provide fundamental knowledge about new functions and expand what we know about microbial interactions in both health and disease, advancing exploitation of the microbiota for our benefit.

DIVERSITY, PHYLOGENY, AND ANTIMICROBIAL NATURE OF BILE ACID CONJUGATION WITHIN THE *LACHNOSPIRACEAE*

Guzior, D.V.;*[1,2] Quinn, R.A.[2]
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Bile acids (BAs), key molecules with fat digestion and homeostatic regulation, are synthesized in the liver and circulate through our upper gastrointestinal tract following a meal via enterohepatic circulation. Conjugation with glycine or taurine is the final step in BA synthesis, performed by the host-associated bile acid-CoA:amino acid N-acyltransferase. BA conjugation was believed to be limited to the host while microbial action on BAs was limited to amino acid deconjugation and hydroxyl group modification (dehydroxylation, oxidation, and epimerization). However, it was recently discovered that gut anaerobes, particularly *Enterocloster bolteae* of the *Lachnospiraceae*, can conjugate a myriad of amino acids to BAs. These microbially conjugated bile acids (MCBAs) represent a fifth mechanism of BA transformation, one that had eluded characterization across over 50 years of research into gut anaerobes and BAs.

Here, we investigated BA conjugation capabilities within the family *Lachnospiraceae* to identify phylogenetic relationships with this unique biochemical function. Members of 10 genera within *Lachnospiraceae* were cultured for 16 hours in the presence of cholate followed by methanol extraction and untargeted LC-MS/MS analysis. Data were submitted to the Global Natural Products Social Molecular Networking database for spectral identification. Here, we identify genera closely related to *Enterocloster* both responsible for and incapable of MCBA production. Genera not associated with the gut (i.e., *Lacrimispora*) did not conjugate cholate. MCBA production was not ubiquitous among gut-associated genera, as *Blautia* species did not have observable MCBA production. However, *Enterocloster*, *Ruminococcus*, and *Lachnoclostridium* species showed robust conjugation including 14 different amino acids and the amino acid precursor citrulline. MCBA producers also displayed a distinct preference for glycine as a substrate, implicating *Lachnospiraceae* in the production of molecular mimics of "host" conjugated bile acids. Little is known about the effects of MCBAs on the host or their microbiome, further demonstrating a need to elucidate the mechanisms behind this novel, widespread, transformation.

July 28-31

Saturday, July 30 — *Clostridioides difficile* Management

1000-1205 Session VIII: *Clostidioides difficile*: Management Update

∗—Indicates Presenter

DIAGNOSIS OF *C. DIFFICILE* INFECTION: NAAT *VS* OTHER DIAGNOSTIC ALGORITHMS, ULTRASENSITIVE TOXIN TESTING

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Although the epidemiology of *Clostridioides difficile* has changed, this organism continues to cause adverse clinical outcomes and increased morbidity, mortality, length of hospital stay, and health-care costs. No single diagnostic test to date has demonstrated optimum sensitivity and specificity for detection of *C. difficile* infection (CDI). Many institutions have embraced multi-step algorithms consistent with guidelines established by various professional societies. Some institutions have successfully improved the pretest probability of molecular assays by implementing appropriate sample rejection criteria and establishing best practice alerts at the time of electronic order entry. Others have established polymerase chain reaction cycle threshold cut-offs to attempt to differentiate symptomatic patients from asymptomatic carriers or to make predictions about severity of disease with variable success. Advances in testing include development of ultrasensitive toxin tests and an expansion of biomarkers that may be more *C. difficile* specific. As research advances our understanding of *C. difficile* pathogenesis and pathophysiology, more information on CDI specific biomarkers is emerging. Finally, assessments of the microbiome and metabolome may expand the diagnostic armamentarium along with advances in mass spectrometry and sequencing technologies.

TREATMENT AND PREVENTION: IDSA/SHEA GUIDELINE UPDATE

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Since publication of the 2017 Infectious Diseases Society of America and Society for Healthcare Epidemiology of America (IDSA/SHEA) clinical practice guidelines for *C. difficile* infection (CDI), new relevant evidence emerged for treatment options in the management of CDI. In 2021 a focused update was published that was restricted to adults and included new data for fidaxomicin and for bezlotoxumab, a monoclonal antibody targeting toxin B produced by *C. difficile.* Both of these agents have increased clinical efficacy and other advantages over older agents, but implementation may be challenging because of initial monetary cost and logistics. Recommendations were developed from a systematic literature review and a rigorous adherence to GRADE (Grading of Recommendations Assessment, Development and Evaluation) methodology with careful attention to wording of the specific recommendations. Conditional recommendations were given for use of fidaxomicin in treatment of initial CDI episodes and recurrent CDI episodes, as well as for adjunctive treatment with bezlotoxumab based on moderate, low, and very low certainty of evidence, respectively. Guideline updates that included treatment recommendations were also published in 2021 by the American College of Gastroenterology (ACG) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Development of the IDSA/SHEA guidelines will be discussed and compared to recommendations by ACG and ESCMID

EFFECT OF THE COVID-19 PANDEMIC ON RATES OF *CLOSTRIDIOIDES DIFFICILE* INFECTION IN ONE VA HOSPITAL AND CORRELATION WITH RATES IN THE NATIONAL VA HEALTHCARE SYSTEM

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Clostridioides difficile infection (CDI) is the most common healthcare-associated infection and is a major global public health concern. In individual patients with COVID-19, hospitalization and antibiotic use may have been expected to increase the risk of CDI, whereas increased COVID-19 infection prevention measures could reduce CDI. As part of the VA Cooperative Studies Program, "CSP#596: Optimal Treatment for Recurrent *C. difficile* Infection (OpTION)," the Hines VA Hospital collects prescreening data on patients with CDI and recurrent CDI (rCDI). We noted a 35% decline in the number of CDI patients in 2020 compared to annual mean over the previous 5 years (2015-2019) at the Hines VA. Although the number of hospital admissions decreased 21% in 2020 vs. 2015-2019 (8293 vs. 10,523), the rate of CDI per 100 admissions also decreased 31% (1.18 vs. 1.72 for the 5 previous years). There was no significant change in the number of outpatients seeking care. The mean rate of rCDI also declined significantly in 2020 compared to the mean over the 5 preceding years (13.3% vs. 21.6%; P = 0.039), representing a 38.4% decline in rCDI in 2020. Changes in patient volume did not fully account for the declines in CDI and rate of rCDI in 2020, which were coincident with the COVID-19 pandemic and societal shutdowns beginning in the spring of 2020. Overall CDI test positivity rate has also declined since the pandemic shutdowns. CDI positivity was 16.6% of tests run in 2018-2019 combined compared to 13.0% in 2020-21 through October of 2021 (P = 0.0026). Similarly, the total number of CDI cases throughout the VA Healthcare System declined by 36.4% during this same period. Despite some pre-pandemic expectations, CDI and rCDI rates decreased significantly during the COVID-19 pandemic.

UPDATE ON IBEZAPOLSTAT CLINICAL TRIAL DEVELOPMENT

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Ibezapolstat (formerly, ACX-362E) is a DNA polymerase IIIC inhibitor in development for the treatment of *Clostridioides difficile* infections (CDI). Ibezapolstat specifically targets low G+C Gram positive bacteria, including some *Firmicutes*. Having completed phase I studies demonstrating a favorable safety and pharmacokinetic profile, phase 2a studies have also demonstrated for the first time effective use of this new class of antibiotics in patients with CDI. In parallel with the clinical trial development, laboratory investigations into the pharmacology and microbiome effects of ibezapolstat are progressing. This presentation will provide updates on the development of ibezapolstat including the ongoing clinical trial and laboratory experiments.

IMPACT OF MISDIAGNOSIS OF *CLOSTRIDIOIDES DIFFICILE* INFECTIONS (CDI) BY STANDARD-OF-CARE SPECIMEN COLLECTION AND TESTING ON ESTIMATES OF HOSPITALIZED CDI INCIDENCE AMONG ADULTS IN LOUISVILLE, KENTUCKY, 2019-2020

Ramirez, J.A.;[1†] Angulo, F.J.;*[2†] Carrico, R.L.;[1] Furmanek, S.;[1†] Oliva, S.P.;[1] Zamparo, J.M.;[2] Gonzalez, E.;[2] Zhang, P.;[2] Wolf Parrish, L.A.;[2] Marimuthu, S.;[3] Pride, M.W.;[4] Gray, S.;[2] Matos Ferreira, C.S.;[2††] Arnold, F.W.;[3] Istúriz, R.E.;[2] Minarovic, N.;[2] Moïsi, J.C.;[2] Jodar, L.[2]

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Background. The incidence of hospitalized *Clostridioides difficile* infection (CDI) is high in the United States, but the impact of misdiagnosis on the reported CDI incidence is uncertain.

Methods. Surveillance identified inpatients ≥50 years-of-age with diarrhea in Louisville, Kentucky, from October 14, 2019, to April 11, 2020. Stool specimens were tested by PCR and cell cytotoxicity neutralization assay (CCNA). A CDI case was a patient with a PCR positive/CCNA positive stool or PCR positive stool with pseudomembranous colitis (PMC). Incidence was adjusted for the hospitalization share of participating hospitals, and, in a sensitivity analysis, for inpatients with diarrhea without a CDI test result. Standard-of-care (SOC) stool specimen CDI testing occurred independent of the study.

Results. Surveillance identified 1541 inpatients with diarrhea, resulting in 109 CLOUD primary CDI cases; 18 (16.5%) had PMC, 36 (33.0%) were admitted to intensive care, and 21 (19.3%) died. The hospitalized CDI incidence in persons ≥50 years-of-age was 154/100 000 population per year (PPY) and, in the sensitivity analysis, 202/100 000 PPY. The SOC hospitalized CDI incidence in persons ≥50 years-of-age was 121/100 000 PPY. Of the 109 CLOUD primary CDI cases, 44 (40.4%) were not SOC-diagnosed (SOC under diagnosis). Of the 75 SOC primary CDI cases that were CLOUD tested, 12 (16.0%) were not CLOUD primary CDI cases (SOC over-diagnosis).

Conclusions. There was a high incidence of hospitalized CDI in persons ≥50 years-of-age, with severe clinical consequences. SOC testing practices resulted in a higher frequency of CDI under diagnosis than over diagnosis. CDI incidence estimates based on SOC testing may be under-estimated.

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Saturday, July 30 **Breakout Lunch Session**

1230-1330 Session IX: Meet the Editors: Tips on Publishing in Peer-Reviewed Journals

SIX-1 Meet the Editors: Tips on Publishing in Peer-Reviewed Journals 50
*Sears, C.L.**

*—Indicates Presenter

MEET THE EDITORS: TIPS ON PUBLISHING IN PEER-REVIEWED JOURNALS

Sears, C.L.*
Johns Hopkins University, Baltimore, MD USA

This session will provide an opportunity to talk to ASA members or attendees with experience in being a journal editor or reviewer. The session is intended as informational for trainees and junior faculty members. Dr. Cynthia Sears, newly appointed Editor in Chief for *The Journal of Infectious Diseases*, Editors of the *Anaerobe Special Issue*, and others will answer your questions and lead the discussion.

Saturday, July 30 — *Clostridioides difficile* Infection

1345-1615 Session X: Basic Science of *Clostridioides difficile* Infection

*—Indicates Presenter

PHENOTYPIC HETEROGENEITY IN *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile has recently been shown to display extensive phenotypic heterogeneity within a clonal population. This heterogeneity arises in part as a result of phase variation by site-specific DNA recombination. This process causes the reversible inversion of short DNA sequences, or 'switches,' which are flanked by inverted repeats and contain regulatory information that impacts expression of neighboring genes. One orientation of the switch favors gene expression and corresponds to the ON orientation, while the other does not and is the OFF orientation. Many *C. difficile* strains develop distinct colony morphotypes: a circular morphology with a smooth perimeter (smooth) and a morphology with filamentous edges (rough). The rough and smooth colonies differ in cell morphology, surface migration, swimming motility, biofilm formation, and virulence. We identified a signal transduction system, which we named CmrRST, that is subject to phase variation and modulates colony morphology and the associated phenotypes. The target(s) of CmrRST regulation that mediate the observed phenotypes remain unknown. Here we discuss progress toward determining how CmrRST controls multiple developmental processes in *C. difficile*.

IMMUNE-MICROBIOTA INTERACTIONS IN DEFENSE AGAINST *CLOSTRIDIOIDES DIFFICILE*

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Fecal microbiota transplantation (FMT) is a successful therapeutic strategy for treating *Clostridioides difficile* infection. Despite remarkable efficacy, implementation of FMT therapy is limited and the mechanism of action remains poorly understood. We sought to determine the contribution of the host immune system in support FMT therapy. Our previous work using a murine *C. difficile*/FMT infection system identified an important role for $CD4^+$ $Foxp3^+$ T-regulatory (T_{reg}) cells in enabling FMT-mediated resolution of *C. difficile* infection. We have extended this work and identified the molecular pathway employed by T_{reg} cells to support FMT. IL-10 expressing $CD4^+$ $Foxp3^+$ T_{reg} cells in the colon expand in frequency and total number following *C. difficile* infection. Further, ablation of IL-10 signaling renders *C. difficile* infected mice non-responsive to FMT therapy. Loss of T_{reg} cells or IL-10 induces a robust type 1 immune response in the colon and activation of T_H1 and type 1 innate lymphoid cells. Amelioration of type 1 inflammation restores the capacity of FMT to resolve *C. difficile* infection. Combined these data support a mechanism by which IL-10 released by T_{reg} cells limits type-1 inflammation in the intestine, thereby supporting an intestinal microenvironment receptive to FMT. These data support the concept that the host's immune status can dictate the success of microbiota-based therapeutics to treat disease.

IMPACT OF THE *C. DIFFICILE* SMALL ACID SOLUBLE PROTEINS ON SPORE PHYSIOLOGY

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Clostridioides difficile is a nosocomial pathogen which causes severe diarrhea and colonic inflammation. *C. difficile* causes disease in susceptible patients when endospores germinate into the toxin-producing vegetative form. The action of these toxins results in diarrhea and the spread of spores into the hospital and healthcare environments. Thus, the destruction of spores is imperative to prevent disease transmission between patients. However, spores are resilient and survive extreme temperatures, chemical exposure, and UV treatment. This makes their elimination from the environment difficult and perpetuates their spread between patients. In the model spore-forming organism, *Bacillus subtilis,* the small acid-soluble proteins (SASPs) contribute to these resistances. The SASPs are a family of small proteins found in all endospore-forming organisms, *C. difficile* included. Although these proteins have high sequence similarity between organisms, the role(s) of the proteins differ. Here, we investigated the role of the main α/β SASPs, SspA and SspB, and two annotated putative SASPs, CDR20291_1130 and CDR20291_3080, in protecting *C. difficile* spores from environmental insults. We found that SspA is necessary for conferring spore UV resistance, SspB minorly contributes, and the annotated putative SASPs do not contribute to UV resistance. In addition, the SASPs minorly contribute to the resistance of nitrous acid. Surprisingly, the combined deletion of *sspA* and *sspB* prevented spore formation. Overall, our data indicate that UV resistance of *C. difficile* spores is dependent on SspA and that SspA and SspB regulate / serve as a checkpoint for spore formation, a previously unreported function of SASPs.

SNEAK ATTACKS: HOST DEFENSE PEPTIDES ENHANCE ANTIBIOTIC EFFICACY AGAINST *C. DIFFICILE* WITHOUT TRIGGERING SPORE FORMATION

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The anaerobic spore-forming bacterium *Clostridioides difficile* is a major cause of nosocomial infections. *C. difficile* is resistant to multiple classes of antibiotics and *C. difficile* infection (CDI) has a very high recurrence rate, making the development of novel therapies against CDI a high public health priority. *C. difficile* persists in the environment and spreads to new hosts in the form of highly resilient, metabolically dormant spores. We have found that the antimicrobial host defense peptides (HDPs) piscidin-1 and piscidin-3 are active against replicative, metabolically active *C. difficile.* Here we report that both piscidins act synergistically with multiple classes of antibiotics with diverse cellular targets. Moreover, while exposure to sub-lethal concentrations of antibiotics stimulates spore formation and may contribute to infection persistence, piscidins kill *C. difficile* without triggering sporulation. HDPs are a promising avenue of enhancing antibiotic efficacy against drug-resistant infections.

COLONIZATION OF HAMSTERS WITH NONTOXIGENIC *CLOSTRIDIODES DIFFICILE* REA TYPE M3 (M3) FOLLOWING TREATMENT WITH FIDAXOMICIN OR VANCOMYCIN

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Oral fidaxomicin is a therapy for *Clostridiodes difficile* infection (CDI), comparable to vancomycin in efficacy and better at preventing recurrence, possibly in part due to residual fidaxomicin in the stool. Colonization of hamsters and humans with M3 has been well-characterized as a measure for prevention of primary recurrent CDI but residual fidaxomicin in stool could inhibit M3 colonization. As M3 prevention of CDI is dependent upon colonization, we compared the ability of M3 to colonize hamsters after treatment with either fidaxomicin or vancomycin.

Groups of 10 hamsters received antibiotic therapy and two control hamsters without therapy were included as M3 colonization controls. All hamsters were given clindamycin on Day 0 at 30mg/kg by oral gavage to disrupt normal intestinal microbiota. On Days 2-6, 10 assay hamsters were given fidaxomicin at 5mg/kg/day by oral gavage for a total of 5 consecutive doses. On Days 7-14, 12 hamsters received M3[#] spores at $1x10^6$ cfu/day by oral gavage for a total of 7 consecutive doses. Fecal pellets were cultured on TCCFA pre- and post-M3 inoculation for the onset and persistence of M3 colonization until the end of study on Day 43. This study was then repeated with vancomycin as the therapeutic antibiotic given at 5 mg/kg per dose.

M3 successfully colonized all hamsters in both studies, with colonization onset occurring in fidaxomicin-treated hamsters at 1-4 days after initial M3 administration and in vancomycin-treated hamsters at 1-3 days after initial M3 administration. One fidaxomicin-treated hamster tested negative for M3 at Day 18 and remained negative, but the others remained positive for M3 until the end of the study. All vancomycin-treated hamsters tested positive for M3 from the time of colonization onset until the end of the study.

We conclude that M3 is an effective colonizer of hamsters treated with either fidaxomicin or vancomycin and is a viable clinical candidate for prevention of recurrent CDI in patients receiving therapy with either antibiotic.

[#]NTCD-M3 spores supplied by Destiny Pharma plc, Brighton, UK

DISTINCT SINGLE-CELL GROWTH DYNAMICS OF *CLOSTRIDIOIDES DIFFICILE* CLADE 5 STRAINS REVEALED BY ANAEROBIC TIME-LAPSE MICROSCOPY

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ST-11 (RT078) *Clostridioides difficile* strains cause severe disease and are responsible for a growing number of community-acquired infections, including transmission between humans and livestock. ST-11 is part of *C. difficile* clade 5, a distinct lineage that is currently undergoing speciation away from *C. difficile* clades 1-4. To date, little is known about the factors that contribute to ST-11's broad host-range. Clade 5 strains have been reported to produce distinct colony morphologies, so we sought to test whether ST-11 strains exhibit distinct growth properties. To test this hypothesis, we developed a novel method for measuring *C. difficile* growth properties over many hours using anaerobic, time-lapse microscopy. By coupling our imaging method with a deep-learning based image analysis pipeline, we discovered striking differences in ST-11 growth dynamics and morphology relative to *C. difficile* strains from clades 1-4. ST-11 isolates grew at twice the rate of clade 1-4 strains on TY media (15 min vs. 30 min doubling time) and typically formed long chains that occasionally approach 500 μm long. Additionally, chained ST-11 cells appear to have a more flexible cell wall, indicating differences in peptidoglycan structure. We also identified a single ST-11 clinical isolate that does not form chains but elongates at a similar rate relative to other ST-11 strains, we are conducting genomic analyses to determine the genetic basis of the chained phenotype. Notably, the differences in growth and morphology we have uncovered may play an important role in the infectious cycle of ST-11 strains. Since our approach uses readily available materials, our work also highlights the potential of our novel time-lapse microscopy method for gaining insight into the physiology of many anaerobes.


Anaerobe 2022

Saturday, July 30 ***Anaerobic Cultivation***

1345-1615 Session XI: *Optimizing Anaerobic Cultivation*

*—Indicates Presenter

REVEALING THE HIDDEN LIFESTYLE OF AN ULTRA-SMALL SACCHARIBACTERIA

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Saccharibacteria (TM7) are members of the large lineage of bacteria termed Candidate Phyla Radiation (CPR). CPR bacteria are characterized by having an extremely small cell size (200-500nm) and a reduced genome (~1 Mbp) lacking multiple essential biosynthetic pathways. Furthermore, Saccharibacteria are detected as a part of multiple healthy human microbiome communities, including oral, gut, and vaginal microbiome. 16S rRNA profiling studies also have shown that they have increased abundance in numerous inflammatory diseases, such as periodontitis, inflammatory bowel disease, and vaginosis. Because of these associations, Saccharibacteria are thought to be opportunistic pathogens. However, due to its recalcitrance to cultivation, no causal research has been conducted to investigate their role in inflammatory diseases. In 2015, first Saccharibacteria strain was cultured from the human oral cavity. Unexpectedly, it grew on another bacteria as an episymbiont, complicating their symbiosis with the human host. Based on this first cultured Saccharibacteria, we developed a host baiting method to culture multiple additional Saccharibacteria species from the human oral cavity. These strains were all episymbionts, growing on the surface of Actinobacteria. To further characterize their pathogenicity, we applied them in a mouse ligature-induced periodontal model. Surprisingly, they reduced inflammation and subsequent bone loss by modulating their host bacterial pathogenicity. Two host bacterial functions involved in collagen binding and utilization of eukaryotic sialic acid were identified as responsible for the host bacterial phenotype change. This down-regulation of host bacterial pathogenicity by Saccharibacteria was shown for multiple Saccharibacteria/host bacterial pairs. Therefore, despite previous belief that Saccharibacteria are pathogenic, they could protect mammalian host from inflammatory damage induced by their host bacteria.

HUMAN MICROBIOTA DIVERSITY THROUGH CULTURE-ENRICHED METAGENOMICS

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The culturing of human-associated microbiomes has increased dramatically in the last few years, and methodologies for comprehensive culturing of these microbiota are resulting in rapidly growing strain collections and high-quality genome assemblies. In parallel, there has been an increase in shotgun metagenomics sequencing of microbial communities. Both of these approaches have advantages and limitations. A complementary approach is to combine comprehensive culturing with metagenomic sequencing – **culture-enriched metagenomics** (CEMG). Here, we use a variety of culture conditions to capture the diversity of the microbiota in a sample including enrichment of lower abundant taxa. Each culture condition is treated as independent "community" and recovered as a plate pool which can be preserved for recovery of specific organisms of interest. Each plate pool, is then subjected to metagenomic sequencing, and the sequences assembled, binned, and annotated. The number of conditions to sequence can be reduced using a plate coverage algorithm to identify the minimum number of plates that capture the diversity cultured.

CEMG has several advantages over direct shotgun metagenomics (DMG). Culturing provides better coverage of taxa that may be at low abundance in the original sample and therefore poorly represented in DMG. The assembled contigs are larger, improving annotation and identification of large gene clusters, which often assemble poorly in DMG data. The pooled assemblies result in significantly improved binning and more metagenomic assembled genomes (MAGs). The MAGs from CEMG tend to be larger than from DMG, because of improved binning.

The depth of coverage and improved assembly across the microbiome being studied results in 3-4 fold greater identification in functions of interest, as well as the ability to find more intact large functional gene clusters. Diversity of glycosyl hydrolases and identification of biosynthetic gene clusters within the human gut microbiome will be used to illustrate the utility of CEMG.

ANAEROBIC CULTIVATION AND STRAIN-BANKING FROM THE HUMAN GASTROINTESTINAL TRACT

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Bacteria comprise the largest proportion of the human gut microbiota and for decades, most enteric bacterial species were thought to be unculturable. In recent years, we and others have successfully isolated thousands of species from the human gut microbiota to enable the study of their unique biology and translation of their therapeutic potential. Live Biotherapeutic Products are medicines in which the active ingredients are viable microorganisms. Microbiotica is discovering and developing drugs made from therapeutic strains of bacteria, leveraging two proprietary, world leading platform resources; the Microbiotica Culture Collection, (MCC) and the Microbiotica Reference Genome Database, (MRGD). The MCC comprehensively represents the phylogenetic diversity typical of the healthy human microbiome and includes substantial taxonomic novelty. The Microbiotica Reference Genome Database (MRGD) contains high quality whole genome sequences for the species archived as pure cultures in the MCC. Together, the MCC and MRGD enable strain-level microbiome profiling with exquisite accuracy and precision. Drug refinement and development is also made possible by access to multiple strains of key therapeutic species. Microbiotica is currently advancing two drugs towards the clinic; one for the treatment of ulcerative colitis and another designed to stimulate cancer patients to respond favourably to immune checkpoint inhibitor therapy.

ENHANCING THE ABUNDANCE OF UNDERSTUDIED FASTIDIOUS SPECIES WITHIN ORAL MICROCOSMS

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The oral microbiome contains hundreds of bacterial species; however, surprisingly little is known about the biology of the vast majority of these organisms. In microcosm cultures, well-characterized saccharolytic species such as the streptococci tend to overwhelm the communities, while slow-growing fastidious organisms survive as small percentages of the remaining bacteria. An exceptionally large number of these less abundant organisms are strongly associated with sites of oral disease, but remain under- or even completely unstudied.

In order to maximize the growth of less prevalent fastidious bacteria and decrease the abundance of prominent species within microcosm cultures, we developed a new culture methodology using oral plaque and abscess clinical specimens as inocula. Our approach combines the use of multiple timepoints with two different growth media, the well-characterized SHI media and a novel low-carbohydrate nutrient-rich growth media, to allow for the enrichment of certain species with disparate growth rates and metabolic preferences. Replicate oral microcosms were cultured for two, four, and six days under anaerobic conditions, with fresh media supplemented every 48 hours. gDNA was extracted using the ZymoBiomics gDNA kit, and 16S sequencing of the V3-V4 region was performed by Zymo Research. Community compositions were analyzed using the DADA2 pipeline. The results indicate that the community structure of time-resolved microcosms shifts from predominantly fast-growing early colonizers, such as the streptococci, towards lower abundance fastidious species, while still retaining a highly similar overall species diversity. Distinct community profiles were also observed between plaque and abscess microcosms using both media. Our results reveal a straightforward, reproducible protocol to enrich less prevalent oral microbiome taxa, while decreasing the proportion of the well-studied dominant organisms. This approach can aid efforts to isolate understudied fastidious oral microbes from clinical specimens as well as support screening studies to detect novel bioactivities from microcosm communities.

AUTOMATED ANALYSIS OF MICROBIAL GROWTH REVEALS PHENOTYPIC DIVERSITY OF *CLOSTRIDIOIDES DIFFICILE*

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

We therefore aimed to identify whether carbon substrate utilization by *C. difficile* isolates explains the distribution of ribotypes in the state of Texas and the novel emergence of ribotype 255. We developed a framework for the rapid analysis of carbon substrate utilization with Biolog Phenotype Microarray carbon source plates and designed a new software, Analysis of Microbial Growth Assays (AMiGA), for modelling microbial growth curves. Using this integrative approach, we profiled clinical isolates collected through an active surveillance network in the state of Texas. Clinical isolates generally clustered by ribotype based on their carbon substrate utilization. Ribotypes dominant in Texas (RT027 and RT014-020) exhibited higher area under the growth curve on carbon substrates commonly metabolized by *C. difficile*. Our analysis identified several substrates, such as leucine, melezitose, sorbitol, and trehalose, that are differentially metabolized by distinct ribotypes. It also showed that ribotype 255 grows faster than most ribotypes on several carbon substrates. Finally, animal-associated ribotypes exhibited higher total growth on simple sugars than human-associated ribotypes. Ongoing work will continue to profile additional isolates and validate substrate-based fitness advantages with genomic verification, molecular characterization, and competition assays.

PSEUDOMONAS AERUGINOSA ISOLATES FROM CHRONIC RHINOSINUSITIS DEVELOP INCREASED VIRULENCE UNDER ANAEROBIC CONDITIONS

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Introduction: Genotypic and phenotypic modifications contribute to the virulence and persistence of *Pseudomonas aeruginosa* infections— a common organism in recalcitrant cystic fibrosis (CF) and non-CF chronic rhinosinusitis (CRS). The objectives of this study are to compare phenotypic characteristics and gene expression patterns of clinical *P. aeruginosa* isolates from CF and non-CF CRS subjects under aerobic and anaerobic culture conditions.

Methods: We analyzed 12 phenotypic characteristics of 28 *P. aeruginosa* isolates [24 clinical isolates (16 non-CF CRS and 8 CF CRS) and 4 laboratory species (PAO1, PA14, PA14ΔacsA, PA14ΔacsAΔprpB] grown under aerobic or anaerobic conditions. RNA-seq of selected samples (3 isolates per group (total six human samples and PA14 lab reference) was performed to identify transcriptomic signatures of *Pseudomonas*. Transcripts were considered to be differentially expressed if their expression values (log2) differed by a factor of 1.5 and FDR<0.05.

Results: CF *P. aeruginosa* isolates were from a significantly younger patient population than those isolates from non-CF (Age, Non-CF=58.9+/-3.8, CF=32.1+/-3.5, $p<0.0005$). Swimming motilities and protease productions were increased in CF compared to non-CF isolates under the aerobic conditions, although they lacked statistical significance. Protease productions were significantly higher when grown under aerobic conditions, and solely CF isolates produced protease under the anaerobic conditions. Under anaerobic conditions, genes related to virulence factors, biofilm formation, and antibiotic resistance were significantly up-regulated compared to species grown aerobically (cut-off: fold change>=+2, $p<0.05$, $q<0.05$). Principal component analysis plots from RNA-seq demonstrated that 1) CF CRS isolates were dissimilar to those isolates from non-CF CRS, and 2) CF isolates grown under the anaerobic conditions were dissimilar to those grown aerobically.

Conclusions: Clinical isolates from CF CRS were highly dissimilar compared to CRS and virulent factors were upregulated when grown under anaerobic conditions. The anaerobic environment of the hypoxic sinus could influence the virulence of *P. aeruginosa* in CRS.

July 28-31

Saturday, July 30 — *Clostridioides diffcile* Hot Topics

1630-1730 Session XII: *Clostridioides diffcile* Hot Topics

⁎—Indicates Presenter

TAURINE CONJUGATED BILE ACIDS AMELIORATE APOPTOSIS CAUSED BY *CLOSTRIDIOIDES DIFFICILE* TOXINS

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Clostridioides difficile infection (CDI) is a highly inflammatory disease mediated by the production of two large toxins, TcdA and TcdB. Toxin-induced inflammation is significantly associated with CDI disease severity and mortality, thus therapies that target the host inflammatory response represent a promising yet unexplored strategy for treating CDI. Several bile acids are known to maintain the integrity of the intestinal epithelium, dampen inflammation and attenuate gastrointestinal disease, yet their cytoprotective properties have not been investigated in CDI. To probe whether bile acids can confer protection against toxin-mediated damage at the cellular level, we assessed the inhibitory activity of bile acids against toxin-induced apoptosis in Caco-2 cells. Caspase activation was assessed in cells treated with TcdA and TcdB and 21 individual host- and microbiota-derived conjugated and deconjugated bile acids. Cells were incubated with toxins for eight hours before the addition of bile acids. This screen revealed that several conjugated bile acids can induce a protective host response against toxins and ameliorate toxin-induced apoptosis. Taurine conjugated ursodeoxycholic acid (TUDCA) yielded the most inhibitory response against toxin-induced apoptosis. TUDCA was protective against both purified TcdA and TcdB, as well as endogenously produced toxins from *C. difficile* supernatant. Interestingly, TUDCA was significantly more protective than its unconjugated form, UDCA. *In vitro*, TUDCA did not inhibit *C. difficile* growth or toxin activity, whereas UDCA significantly reduced toxin activity in a Vero cell assay while slightly increasing *C. difficile* growth after 24 hours. These results demonstrate that bile acid conjugation can have profound effects on *C. difficile* as well as the host and that conjugated and unconjugated bile acids may exert different therapeutic mechanisms against CDI. Future work will identify of the host cell pathways that are altered by TUDCA in order to reveal protective mechanisms that can potentially be leveraged as a host-directed therapy for treating CDI.

OLFACTOMEDIN-4 EXACERBATES *CLOSTRIDIOIDES DIFFICILE* INFECTION-INDUCED MORTALITY IN MICE

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Clostridioides difficile is the most common cause of nosocomial infectious diarrhea in the western world. Toxins (A, B, and binary toxins) generated by *C. difficile* bacteria induce damage to intestinal epithelial cells, including crypt stem cells. The damage to the crypt stem cells is shown to perturb the host's capacity to repair the epithelial injury, delaying disease resolution. Olfactomedin-4 (OLFM-4) is a glycoprotein expressed in intestinal crypt stem cells, and its increased expression in the colon is associated with ulcerative colitis, Crohn's disease, and *Helicobacter pylori* infection. However, the effect of OLFM4 on *C. difficile* infection (CDI) pathogenesis remains unknown.

We examined the role of OLFM4 in CDI using wildtype (WT) and OLFM4 deficient ($OLFM4^{-/-}$) mice. Our data reveal that compared to WT mice, $OLFM4^{-/-}$ mice exhibited: ***(i)*** faster resolution of diarrhea; ***(ii)*** better survival; and ***(iii)*** more tissue and systemic eosinophils after CDI. In WT mice, we found that: ***(iv)*** $OLFM4^{+}$ cells preferentially aggregate to areas of injured epithelium; and ***(v)*** the number of $OLFM4^{+}$ cells in cecal tissue correlate with higher epithelial damage score, independent of *C. difficile* bacterial and toxin burden. Notably, previous studies show that higher tissue and systemic eosinophils are associated with better CDI outcomes. Therefore, our data in conjunction with published literature suggest that OLFM4 worsens CDI by delaying eosinophil-driven protective gut responses. Our studies are now focused on determining the underlying mechanisms by which lack of OLFM4 is protective in CDI. Defining the functional role of OLFM4 in CDI and its effects on eosinophil recruitment and stem cell-mediated repair has the potential to identify novel host targets for CDI therapeutics.

SEX-MEDIATED DIFFERENCES IN MURINE *CLOSTRIDIOIDES DIFFICILE* INFECTION OUTCOME

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Epidemiological studies have shown that women are at higher risk than men of developing *Clostridioides difficile* infection (CDI). Similar to humans, female mice developed more severe CDI than males when challenged with *C. difficile* strain 630 spores. To study the effect of estrus cycle on CDI, we challenged mice with *C. difficile* spores at different stages of the murine estrus cycle. We then scored animals for CDI severity while monitoring their estrus cycle.

The resulting data was analyzed two different ways: Firstly, we separated mice into four groups (proestrus, estrus, metestrus, or diestrus) depending on their estrus stage at the day of challenge. Animals were kept in their initial group even if their estrus cycle stage changed in the days post-challenge. This binning allows to analyze the effect of each estrus cycle stage on infection onset. Using this method, we found that female mice that are in the estrus phase during the early stages of infection develop less virulent CDI compared to animals that are in the metestrus phase.

In a second analysis, we put mice into the same four groups, but starting at day 1 post-challenge. Animals were moved daily to different estrus groups as their cycle stage changed. This allows to assess the effect of estrus cycle stages on infection progression. We found significant protection from CDI by the proestrus stage at day 2 post-infection. However, this protection disappeared by day 3. Comparison of these two analyses suggests that the estrus cycle strongly affects CDI onset, but has only a minor impact in delaying infection progression.

We have previously shown that CaPA, an aniline-substituted bile salt, prophylactically protected rodents from hypervirulent *C. difficile* strain R20291. To determine the effect of sex on CDI prophylaxis, we challenged male and female mice with *C. difficile* strain R20291 spores. A subset of infected animals were dosed with CaPA. As before, we found that CDI from a hypervirulent strain was milder in untreated male mice than in female mice. Interestingly, CaPA was able to completely protect female mice, but not males, from CDI caused by *C. difficile* strain R20291.

THE DIFFICILE GENOMICS SEQUENCING AND TYPING SERVICE

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The development and delivery of a real time national typing service for *Clostridioides difficile* utilising whole genome sequencing – the Difficile Genomics Sequencing and Typing Service (DIGEST).

C. difficile infection (CDI) remains a priority for healthcare institutions worldwide. The ability to distinguish between transmission, re-infection, and recurrence is key to instigating the appropriate infection prevention and control (IP&C) response.

Originally developed in the UKARU, PCR ribotyping is now the most commonly used method for typing *C. difficile* across Europe. However, newer methods that use whole genome sequencing (WGS) provide far higher discriminatory power and allow us to examine the ecology of *C. difficile* within our healthcare systems with increased certainty.

The Welsh Government and Public Health Wales have prioritised the utilisation of genomics technology to influence public health in Wales with the development of services for several key pathogenic viruses and bacteria, including *C. difficile.*

The DIGEST service includes centralised culture and sequencing of all first line positive stool samples from symptomatic patients from across Wales, within the UKARU and Pathogen Genomics Unit (PENGU). The in-house bioinformatics pipelines developed allow comparison of sequences and clustering of cases down to the zero single nucleotide polymorphism (SNP) level, with results delivered in real time. IP&C teams receive relatedness information via ICNET (clinical surveillance software), whilst the wider clinical teams can visualise additional information, including prescribing history and patient movements via Tableau (business intelligence and analytics software).

The DIGEST service has already proven invaluable in tracking transmission events and has revealed that the ecology and transmission of *C. difficile* is more complex than previously conceived. Health Protection teams within Wales are currently developing resources to investigate inter-institutional transmission events.

July 28-31

Saturday, July 30 **Genetic Manipulation**

1630-1730 **Session XIII:** ***Genetic Manipulation of Anaerobes: Strategies and Successes***

IN PURSUIT OF A *FUSOBACTERIUM* 'PAN-GENETIC' SYSTEM

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Fusobacterium are oral, Gram-negative, anaerobic, opportunistic pathogens that are implicated in the progression and severity of a growing number of tissue infections and cancers. Despite their medial importance, our understanding of distinct roles for *Fusobacterium* in disease have been severely hindered by their genetically recalcitrance. Here, we demonstrate a path to overcome these genetic barriers in *Fusobacterium nucleatum* by using native DNA methyltransferases as a host mimicry strategy to bypass R-M system cleavage of user introduced plasmid DNA. I will present these proof of concept studies with the goal of providing a molecular microbiology roadmap to usher in a new era of virulence gene identification to accelerate our understanding of how these opportunistic pathogens drive host-affecting processes including cellular invasion, DNA damage, proinflammatory signaling, and immune system modulation in disease.

SYSTEMATIC DESIGN OF GENETIC SYSTEMS FOR NON-MODEL ORGANISMS TO ADVANCE FUNCTIONAL INTERROGATIONS OF THE HUMAN MICROBIOTA

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Genetic engineering is a powerful approach for discovering fundamental aspects of bacterial physiology, metabolism, and pathogenesis. However, the full power of genetic engineering can only be applied to a few model organisms. Biological diversity and strain-level variation in restriction-modification (RM) systems are critical barriers keeping most bacteria beyond the full potential of genetics. We previously designed an approach to evade RM systems during construction of genetic tools. We now leverage pangenome/pan-epigenome analyses of single species strain collections (in particular anaerobic species) isolated from multiple-niches across the human microbiota to rationally inform the design, creation, and implementation of genetic systems in non-model organisms relevant to human health and disease.


Anaerobe 2022

Sunday, July 31 **Emerging Anaerobes**

0800-1000 Session XIV: Emerging Anaerobes and Disease Associations

THE *VEILLONELLACEAE:* AN EMERGING PATHOGENIC GENUS WITH CHANGING SUSCEPTIBILITY PATTERNS

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An increasing number of *Veillonella* species (12 to 14) are being recovered, most of which cannot be identified via conventional biochemical and phenotypic methods, including an increasing number from human infections. These lactate utilizing (conversion to propionate and acetate by the methylmalonyl-CoA pathway) Gram-negative cocci may be normal components of the oral and intestinal flora in mammals but also have been etiological agents in endocarditis, discitis, osteomyelitis, sepsis and from tongue biofilms associated with halitosis. Of 305 *Veillonella* isolates in our collection, 118 had "no good match" and 141 were identified by PCR gene-sequencing. Our lab identified 5 different species, plus 11 "no-species match" isolates from clinical specimens obtained from multiple body sites during the years 1984 to 2017, and tested them against 9 antimicrobial agents. *V. parvula* was the species most readily identified using Rapid ANA kits, and the most frequently identified species (93/141, 65.9%). It was recovered from abdominal infections (22), respiratory (15) blood (11) human bites (12) and multiple tongue cultures (as part of a halitosis study). Other commonly isolated *Veillonella* sp. were *V. atypica* (19), *V. dispar* (13), *V. rogosae* (4) and *V. tobetsuensis* (1). Breakpoints for piperacillin-tazobactam (P-T) CLSI (>64 µg/ml) and EUCAST (>16 µg/ml) differ making the interpretation of the literature problematic. Overall resistance was exhibited against P-T (10% CLSI vs. 44% EUCAST) and penicillin-G (46% both). There was considerable resistance to moxifloxacin (27% CLSI, no EUCAST breakpoint) and doxycycline (23% CLSI, no EUCAST breakpoint). Moxifloxacin resistance of *V. parvula* rose from 15% (1984-2000) to 39% (2001-2017) in *V. dispar (11% to 50%)* and *V. atypica (*29% to 42%). Resistance was more likely in abdominal isolates, (P-T, 13% CLSI vs 50% EUCAST; moxifloxacin, 30% CLSI ; and doxycycline (23% CLSI) , respiratory (P-T, 10% CLSI vs 50% EUCAST; moxifloxacin, 30% CLSI; doxy, 20% CLSI), and blood cultures (P-T 12% CLSI vs 29% EUCAST; moxifloxacin, 24% CLSI; and doxycycline 12% CLSI) tended to be more resistant than those recovered from other sources. No meropenem and rare metronidazole (1 isolate) resistance was encountered. *V. parvula* exhibited sporadic resistance to ampicillin-sulbactam and linezolid

NOVEL ANAEROBES IN THE REPRODUCTIVE TRACT

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The human vagina is unique among mammals in having low vaginal pH and being colonized with an abundance of lactobacilli. In contrast, during the dysbiotic condition bacterial vaginosis (BV), there is an increase in vaginal pH and the vagina is colonized with diverse anaerobes. Characterization of the human vaginal microbiota using molecular approaches (16S rRNA gene sequencing) demonstrated the presence of many novel anaerobes that were uncultivated at the time of discovery. Interestingly, many anaerobes detected in the human vagina using molecular techniques have also been detected in non-human primates (NHPs) including *Prevotella* and *Porphyromonas* spp., and the novel bacterial vaginosis-associated bacterium-2 (BVAB2). However, lactobacilli are not abundant in NHPs, and lactobacilli such as *Lactobacillus crispatus* and *Lactobacillus iners* typically noted in the human vagina are not routinely detected in NHPs. Recent cultivation efforts have facilitated the propagation of over 100 bacterial species from the human vagina, including many novel bacteria. Having cultivated human vaginal bacterial isolates that can be manipulated in the laboratory provides opportunities for understanding microbial interactions and pathogenesis of BV. Systematic biochemical, phylogenetic, and genome characterization of these novel human vaginal bacteria, along with deposition of cultured type strains in public repositories is facilitating valid publication of names for these bacteria. One example is *Mageeibacillus indolicus* (formerly BVAB3), a member of the *Oscillospiraceae* family. *M. indolicus* was isolated both from the human vagina and endometrium and has been shown to be associated with BV and cervicitis. Another example includes novel Megasphaera species (*M. lornae, M. hutchinsoni, M. vaginalis*) which are members of the *Veillonellaceae* family. Megasphaera species are useful for diagnosing BV and have been associated with adverse outcomes such as higher risk for preterm birth and HIV acquisition. Other members of the *Veillonellaceae* that have been isolated from the human vagina include several *Veillonella* and *Dialister* species, including the novel *Dialister* sp. type 2. In this talk, I will discuss our approaches to propagate and characterize novel human vaginal bacteria.

MECHANISMS OF VIRULENCE AND PROTECTION IN *PAENICLOSTRIDIUM SORDELLII* INFECTION

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Human infections caused by the toxin-producing, anaerobic and spore-forming bacterium *Paeniclostridium sordellii* are associated with a treatment-refractory toxic shock syndrome (TSS). Reproductive-age women are at increased risk for *P. sordellii* infection (PSI), because this organism can cause intrauterine infection following childbirth or abortion (spontaneous, therapeutic, or illicit). PSI-induced TSS in this setting is nearly 100% fatal, and there are no effective treatments. *P. sordellii* secretes cytotoxins that are similar in structure and function to toxins generated by the related pathogen *Clostridioides difficile*: lethal toxin (TcsL), similar to *C. difficile* TcdB, and hemorrhagic toxin (TcsH), similar to *C. difficile* TcdA. Neutralizing the cytopathic effect of TcsL by using a recombinant anti-TcdB antibody that is used for *C. difficile* might protect humans against toxic shock caused by TcsL-expressing *P. sordellii.* In cell culture, we characterized two anti-TcdB monoclonal antibodies (mABs), PA41 and CDB1, via neutralization assays and found that both mABs could significantly neutralize the cytotoxic activity of recombinant TcsL. We next sought to determine the efficacy of PA41 and CDB1 *in vivo*. However, developing effective interventions against PSI (and TSS) is stymied by a lack of animal models of intrauterine infection and an incomplete understanding of how *P. sordellii* induces disease in this setting. To address this problem, we developed an innovative mouse model system in which to study PSI using a transcervical inoculation method. Our model produces a disease that more closely represents the nature of PSI in postnatal and post-abortive women and allowed us to further study this potential antibody therapy against PSI. The results of the *in vivo* mAB neutralization studies involving TcsL intoxications and *P. sordellii* infections will be presented.

SNEATHIA VAGINALIS: AN EMERGING PATHOGEN OF PREGNANCY, AND ITS PORE-FORMING TOXIN

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The purpose of this study was to localize the pore-forming domain within the cytopathogenic toxin A of *Sneathia vaginalis. S. vaginalis* is a fastidious gram-negative anaerobe that has emerged only recently with the increased use of DNA sequencing-based bacterial identification. It is frequently identified in amniotic fluid and is associated with preterm birth and premature rupture of membranes. The biology and virulence determinants of the organism are currently very poorly understood. Our lab has focused efforts on the characterization of *S. vaginalis* pathogenesis, and we have made significant strides in the development of tissue culture, placental explant, and mouse models. We identified a pore-forming toxin produced by *S. vaginalis* that lyses human epithelial cells and red blood cells (RBC), and we named the toxin CptA for cytopathogenic toxin, component A. Rabbit antiserum against CptA neutralizes the pore-forming activity and prevents *S. vaginalis* from traversing human fetal membranes suggesting that CptA plays an important role in pathogenesis.

Methods: Structural and functional prediction of CptA was performed using in silico bioinformatics tools including GlobPlot, iTasser, and PSIPRED, and based on predictions, a series of truncated CptA peptides were expressed in *E. coli.* We assessed function of the peptides through binding assays, hemolysis assays, and epithelial cell permeabilization.

Results: The amino-terminal two thirds of CptA adhered to human epithelial cells and formed pores in epithelial cells and red blood cells while the carboxy-terminal third of the toxin was unable to permeabilize human cells.

Conclusions: *S. vaginalis* is poorly understood, but has been strongly linked to infection and poor pregnancy outcome. A better understanding of its role and pathogenesis could promote the development of new modes of intervention to prevent infectious preterm birth. This study advances knowledge about the only known *S. vaginalis* virulence factor, CptA and demonstrates that the aminoterminal portion is necessary and sufficient for the pore-forming activity of the toxin.

GARDNERELLA DIVERSITY AND ECOLOGY IN PREGNANCY AND PRETERM BIRTH

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Gardnerella spp. are linked to suboptimal microbiome community structure and negative health outcomes, but are also found in healthy vaginal microbiomes. Many microbiome analysis methods treat all *Gardnerella* spp. as one species. We *hypothesize* that *Gardnerella* clades and genomic species differentially impact microbiome composition and are differentially associated with preterm birth.

Shotgun metagenomic sequencing data collected longitudinally from three cohorts of pregnant women were assessed (Stanford Enriched: n=62 samples; UAB Enriched: n=45; MOMS-PI: n=781). The Stanford and UAB Enriched cohorts were enriched in *Gardnerella*, based on previously performed amplicon sequencing. A fourth cohort was subset from the MOMS-PI cohort (MOMS-PI Enriched; n=145) to mimic the sampling of the Stanford and UAB cohorts. Relative abundance of *Gardnerella* clades and genomic species and other taxa was quantified. Sample bacterial load in was defined as the ratio of bacterial shotgun reads to human shotgun reads. Associations between *Gardnerella* variant presence and bacterial load and co-occurrence of other taxa were measured. Subject average *Gardnerella* abundance and preterm birth was assessed.

Gardnerella clades were differentially associated with signatures of the vaginal microbiome. The genus *Gardnerella* was associated with increased bacterial load, but not all clades were associated with increased bacterial load. Samples often contained multiple *Gardnerella* variants, but the number of clades per sample varied among cohorts and was associated with increased bacterial load. Taxon co-occurrence patterns matched previously described community structures, but variation among *Gardnerella* clades was observed. *Gardnerella* was not associated with preterm birth; selection for enrichment in *Gardnerella* likely prevented replicating this previously found association.

Gardnerella variants differentially impact the vaginal microbiome. The ability to accurately resolve *Gardnerella* variants is important to understand the impacts of *Gardnerella* on the microbiome, and determine which variants indicate health risks.

GLYCOGEN BREAK DOWN BY VAGINAL BACTERIA

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The vaginal epithelium is rich in the polysaccharide glycogen, a major source of carbon and energy for vaginal bacteria. Few studies have investigated the amylolytic proteins bacteria encode or their ability to convert glycogen into fermentable sugars.

We searched the genomes of diverse vaginal bacteria for predicted glycoside hydrolases which might degrade glycogen. We used Phobius to predict which proteins are secreted and have access to extracellular glycogen. We identified candidate genes in the genomes of *Gardnerella, Lactobacillus, Mobiluncus, Peptoniphilus,* and *Prevotella*. Many of these proteins were likely anchored to the cell envelope, but some, including a protein unique to *Gardnerella swidsinskii,* appeared to be completely secreted.

To confirm our *in silico* analysis, we developed an *in vitro* assay to measure the rate of glycogen degradation for both surface-attached and secreted amylases. Bacteria were grown on solid media, then suspended with their secreted proteins in physiological saline. The suspension was incubated anaerobically at 37°C with a known concentration of bovine liver glycogen. Post incubation, cells were centrifuged and the concentration of glycogen remaining in the supernatant quantified by measuring color change after addition of Lugol's Iodine. The rate of degradation was normalized with colony forming units present in the cell suspension.

Using this assay, we found species of *Lactobacillus, Gardnerella,* and *Prevotella* that were capable of degrading glycogen, with rates varying by two orders of magnitude. *Gardnerella swidsinskii* was the fastest degrader we tested. Although secreted glycoside hydrolases appear to be more common among vaginal isolates of *Lactobacillus crispatus* than isolates from other body sites, vaginal *Lactobacillus crispatus* degraded glycogen too slowly for our assay to detect at pH 7. *Lactobacillus iners,* however, degraded glycogen at a comparable rate to *Gardnerella vaginalis*.

Our results confirm that vaginal bacteria are capable of degrading glycogen. The efficiency at which they break down the polysaccharide varies widely by species, which may have implications for community dynamics in the vaginal microbiota.


Anaerobe 2022

Sunday, July 31 Anaerobes in Low Biomass Environments

1015-1215 Session XV: Anaerobes in Low Biomass Environments

INTRO TO THE CHALLENGE: THE PLACENTAL MICROBIOME?

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Molecular microbiological methods have detected and identified numerous anaerobes and other microbes present in the human microbiome, particularly at mucosal surfaces such as the mouth, gut, skin, and vagina. These same molecular approaches have been applied to detect microbes in low biomass sites such as blood, placenta, and internal organs that are usually considered sterile. However, this application poses several technical challenges, and necessitates the adoption of appropriate experimental controls to assure that the microbes detected truly reflect colonization. I will discuss the debate over whether the human placenta has a resident microbiome, elaborate the experimental controls that are optimal for studying the microbiome of low biomass environments, and introduce a study that sought to determine if the female upper reproductive tract has a resident microbiota.

FALLOPIAN TUBE MICROBIOME ANALYSIS: POTENTIAL PITFALLS

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The fallopian tube (FT) is the site of origin for a large percentage of ovarian cancers, and is the conduit for the ascending infections in pelvic inflammatory diseases (PID). Several studies indicate that the female upper genital tract including the fallopian tubes may not be sterile. However, there are many limitations with these studies and the findings were inconsistent. In this prospective study, we collected swabs from the fallopian tubes and other surgical sites as controls in sterile fashion to profile the microbiota in the fallopian tubes.

After obtaining informed consent from patients, we collected swabs from the fallopian tubes, ovarian surfaces, paracolic gutters, laparoscopic ports, and sham swabs (air in the operating room) in sterile fashion. Surgical indications included ovarian cancers of various histological types, prophylactic salpingo-oophrectomies due to germline BRCA or other mutations, benign gynecological disorders such as ovarian cysts or endometriosis. DNA was extracted from the swabs and V3-V4 region of 16S rRNA gene was amplified and sequenced, and the bacterial concentrations were quantified using qPCR. A total of 208 patients were enrolled and 1008 swabs were processed. The bacterial concentrations of fallopian tube and ovarian surfaces consistently ranged 10~1000 copies of 16S rRNA genes/ul of DNA. Over 700 bacterial species were identified in the raw sequencing data. Multiple negative controls and filtering approaches were used during the sample collection and data analysis in order to differentiate FT microbiota from the likely contaminants. In this presentation, we will discuss these approaches, and the lessons we learned from conducting a microbiome study in a low-biomass environment.

POINT-COUNTERPOINT DEBATE: ANAEROBES IN THE LUNG: CYSTIC FIBROSIS—ANAEROBES AS PATHOGENS

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Culture-independent bacterial profiling of cystic fibrosis (CF) lower airway samples (e.g. sputum and bronchoalveolar lavage) has revealed complex polymicrobial communities and changing structure of these communities over time in patients. These communities are composed of the primary CF pathogens (such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia*, *Achromobacter* and *Mycobacterium abscessus*) and organisms considered commensals of the oral and nasal passages. These "non-pathogenic" bacteria include obligate and facultative anaerobes. While oral contamination during sample collection is an issue, numerous studies have provided strong evidence that these complex communities are established in the lower airways. Indeed, an increased burden of anaerobes in lower airway samples is hallmark of chronic airway disease (not just CF).

It should also be noted that culturing approaches identified anaerobes in lower airway samples of CF patients over 30 years ago, and all members of the lower airway microbiome identified by molecular methods can be readily cultured using straightforward methods, with very few exceptions. Culture-dependent and culture-independent characterization of these samples are qualitatively similar. However, quantitative culture often provides a very different perspective than culture-independent data and is more sensitive for measuring short term dynamics in microbial populations. Accumulation of cell free DNA (from dead host and microbial cells) and the lack of accurate taxonomic resolution of partial 16S sequencing confounds the culture independent methods.

This talk will address the potential significance of the complex polymicrobial communities in CF airways. Most interactions in ecosystems are neutral and these other organisms present in the airways may just be "opportunistic colonizers," with no impact on disease. However, I will argue that indirect evidence suggests otherwise. Most of the current approaches used to study the CF microbiome are inadequate to address these issues. However, understanding the contribution of the non-traditional pathogens in airway disease will provide new opportunities for a more personalized treatment approach for the management of pulmonary exacerbations in CF.

POINT-COUNTERPOINT DEBATE: ANAEROBES IN THE LUNG: CYSTIC FIBROSIS—ANAEROBES AS COMMENSALS AND TRANSIENTS

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Decades of study using culture-based methods suggest that the lungs of people with cystic fibrosis (CF) are uninfected at birth, and that over time transient and then permanent infections develop. Importantly, a restricted group of organisms (called "CF pathogens") have been thought responsible for infection and epidemiological and clinical observations associate these organisms with disease.

Recent work using DNA sequencing to identify bacteria in CF airways have upended this established model. DNA-based methods suggest that diverse communities of bacteria that were previously considered oral organisms dominate lungs before CF pathogens appear, and co-exist with CF pathogens after CF pathogens become dominant. DNA-based analyses also suggest that the diversity of lung microbiota may be a key disease parameter.

Reconciling the new ideas suggested by DNA-based methods with the established pathogenesis model is challenging as both culture and DNA-based approaches have significant, and in some ways opposing, limitations. Culture analysis is based on the premise that infection is generally caused by a single or small number of pathogens that can be isolated in pure culture. Thus, clinical laboratories selectively culture samples and filter results to remove organisms not considered pathogenic. This could be problematic as culture-based analysis could fail to identify infecting organisms.

In contrast, DNA-based methods cast a wider net, identifying culturable and unculturable organisms, and DNA from live and dead cells without filtering for contaminants. This could be problematic because many of the organisms detected by sequencing are highly abundant in oropharyngeal secretions that contaminate airway samples. Contamination is particularly problematic for throat and sputum samples, and when lung bacterial biomass is low (e.g. early in disease).

This talk will discuss challenges inherent to identifying the composition of lung microbiota due to oropharyngeal and reagent contamination and low bacterial biomass. Overcoming these challenges is critical because future progress depends upon accurately determining which bacteria are present in the lungs of people with CF and contribute to disease.

MAPPING THE INTRATUMORAL MICROBIOTA IN HUMAN ORAL AND COLORECTAL CANCERS

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Within human tumors, cancer cells are surrounded by a complex network of non-malignant cells, which include the intratumoral microbiota. By adapting and applying spatial technologies and single cell RNA sequencing, we uncover host-bacterial spatial and cellular interactions within the tumor microenvironment of two cancer types at the extremes of the gastrointestinal tract, oral, and colorectal cancers. We develop and apply INVADEseq (Invasion Adhesion Directed Expression sequencing) to patient oral squamous cell carcinoma of the tongue and reveal that *Fusobacterium* and *Treponema* species are the dominant cell-associated bacterial taxa within these tumors, predominantly infecting cancer epithelial cells and myeloid cells. Moreover, we show that intracellular bacteria significantly alter transcriptional pathways of infected immune and epithelial cells. Overall, through spatial and single cell analysis, we demonstrate that the intratumoral microbiota impact epithelial and immune cell function supportive of cancer progression in human oral and colorectal cancer.

Sunday, July 31 **One Health**

1015-1215 Session XVI: One Health: Anaerobes in Humans, Animals, and the Environment

NEW INSIGHTS INTO SPORULATION AND ENTEROTOXIN PRODUCTION BY *CLOSTRIDIUM PERFRINGENS* TYPE F

Mehdizadeh Gohari, I.;[1] Li, J.;[1] Navarro, M.A.;[2,3] Mendoca, F.D.;[2] Uzal, F.A.;[2] McClane, B.A.*[1]
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During diseases such as food poisoning and antibiotic-associated diarrhea, *Clostridium perfringens* enterotoxin (CPE) is produced when *C. perfringens* type F strains sporulate in the intestines. *C. perfringens* lacks the phosphorelay that phosphorylates Spo0A to initiate sporulation in *Bacillus spp.* Instead it is generally believed that *C. perfringens* uses one or more of its seven orphan histidine kinases to phosphorylate Spo0A and initiate sporulation. We previously showed (1) that the CPR0195 orphan kinase, but not the CPR1055 orphan kinase, is important for initiating sporulation and CPE production when type F strain SM101 is grown in modified Duncan-Strong sporulation (MDS) medium. In the absence of a good small animal model for studying *C. perfringens* sporulation, we developed an *ex vivo* sporulation model using mouse intestinal contents (MICs) and used it to test sporulation and CPE production by our CPR0195 and CPR1055 kinase mutants in a more pathophysiologic context. Surprisingly, both mutants still sporulated well and produced wild-type CPE levels in MICs. Therefore, we constructed SM101 null mutants unable to produce each of the remaining 5 orphan kinases. Four of these single kinase mutants showed less sporulation and CPE production when grown in MDS; however, only the mutants unable to produce the CPR1953 or CPR1954 kinase showed significantly reduced sporulation and CPE production in the MIC model. These phenotypes were reversible by complementation. Characterization studies showed that the CPR1953 and CPR1954 null mutants produce less Spo0A compared to wild-type SM101. In addition, we showed that, without the membrane binding domain, purified CPR1953 and CPR1954 can directly phosphorylate purified Spo0A *in vitro*. These studies highlight the importance of using pathophysiologically-relevant models to understand *C. perfringens* sporulation and CPE production in a disease context. Since RT PCR results showed that the CPR1953 and CPR1954 kinase genes are still expressed in the absence of one other, these results also indicate that *C. perfringens* requires two orphan kinases for efficient sporulation and CPE production in disease-relevant conditions. The reason for this two kinase requirement will require further study.

Reference: 1) Freedman JC, Li J, Mi E and BA McClane. 2019. Identification of an Important Orphan Histidine Kinase for the Initiation of Sporulation and Enterotoxin Production by *Clostridium perfringens* Type F Strain SM101. mBio. Jan 22;10(1):e02674-18.doi: 10.1128/mBio.02674-18. PMCID: PMC6343041.

GENOMIC AND EVOLUTIONARY INSIGHTS INTO *C. DIFFICILE:* THE QUINTESSENTIAL ONE HEALTH PATHOGEN

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Clostridium (Clostridioides) difficile infection (CDI) remains a significant global One Health threat. The enormous genetic heterogeneity seen across the species underscores its wide ecological versatility and sympatric lifestyle. Moreover, it has driven changes in CDI epidemiology seen in the last 2 decades e.g. increasing rates of community disease with links to *C. difficile* reservoirs outside the hospital setting, e.g. animals and the environment.

This talk will recap recent work exploring the hypothesis that *C. difficile* comprises a complex of distinct species divided along the major evolutionary clades. Whole-genome average nucleotide identity (ANI), pangenomic and Bayesian analyses were used to explore an international collection of over 12,000 *C. difficile* genomes spanning the eight currently defined phylogenetic clades. Major taxonomic incoherence and clear species boundaries separating the three cryptic clades I-III into three novel genomospecies were found. The emergence of these three independent genomospecies predates clades 1-5 by millions of years, rewriting the global population structure of *C. difficile* and the taxonomy of the *Peptostreptococcaceae*. Divergence was likely due to a separation in their habitats or hosts, as the new genomospecies possessed genetic loci, which may allow them to thrive in different ecological niches. Also, these genomospecies show unique and highly divergent toxin gene architecture (which may escape current diagnostic tests), advancing our understanding of the evolution of *C. difficile* and close relatives.

MICROBIOME THERAPY IN COMPANION ANIMALS: STRATEGIES FOR THE CREATION OF ANAEROBIC MICROBIAL COMPLEXES

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Anaerobic bacteria play a key role in clinical microbiology and are implicated in a wide variety metabolic disease. Fecal microbiota transplantation (FMT) has been demonstrated as a curative treatment for chronic gastrointestinal illnesses caused by bacterial overgrowth associated with inappropriate antibiotic therapy in both humans and animals. While its efficacy is robust, the mechanism of action of FMT has not yet been fully elucidated. The development of controlled consortia or "complex" of anaerobic bacteria will allow for *in vivo* study, which could further characterize the therapeutic properties and control the microbiome therapies.

In this work we established a curated healthy reference set for domestic cats and dogs culled from a large database (>15,000 samples) using 16S rRNA sequencing with metadata reported by pet owners. We determined the core microbiome taxa and their ranges in healthy pet cats and dogs. For cats, the core taxa were defined as those that occurred with a prevalence of 75% in the population OR had a minimum median relative abundance of 1.5% and a minimum of 40% prevalence. For dogs, the core taxa were defined as those that occurred with a prevalence of 66% in the population OR had a minimum median relative abundance of 1% and a minimum of 45% prevalence. The core taxa for cats includes members of the genus *Ruminococcus*, *Bacteroides*, *Blautia*, *Catenibacterium*, *Clostridium*, *Collinsella*, *Dialister*, *Faecalibacterium*, *Lachnoclostridium*, *Megamonas*, *Negativibacillus*, *Peptoclostridium*, *Phascolarctobacterium*, *Prevotella* and *Sutterella*. The core taxa for dogs includes members of the genus *Blautia*, *Ruminococcus*, *Fusobacterium*, *Ruminococcus*, *Bacteroides*, *Megamonas*, *Sutterella*, *Peptoclostridium*, *Catenibacterium*, *Faecalibacterium*, *Alloprevotella*, *Phascolarctobacterium* and *Clostridium*. From these core taxa, we develop a targeted list of bacteria and use specifics culture strategies through the culturomics method and isolate a significant number of these anaerobes from healthy pets' samples. This core microbial taxa identified and isolated opens interesting prospects towards the understanding of microbial interaction, along with constructing microbial complexes for controlled microbiome therapies.

EVALUATING THE ROLE OF PORE FORMATION IN *CLOSTRIDIUM PERFRINGENS* ENTEROTOXIN PERMEABILITY EFFECTS

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Clostridium perfringens enterotoxin (CPE) produced by type F strains causes diarrhea and lethal enterotoxemia where CPE is absorbed from the intestinal lumen into the circulation. CPE action involves receptor-binding, oligomerization into a prepore, and pore formation. This study evaluated if pore formation is necessary for CPE to cause *in vitro* and *in vivo* permeability effects using several recombinant CPE (rCPE) species: rCPE and $rCPE_{C186A}$ (which form pores), rC-CPE and $rCPE_{D48A}$ (which bind to receptors but do not oligomerize), $rCPE_{C186A/F91C}$ (which binds and oligomerizes without pore formation) and $rCPE_{Y306A/L315A}$ (which has reduced receptor binding ability). On Caco-2 cells, i) rCPE and $rCPE_{C186A}$ were cytotoxic while the other rCPE species were not, ii) rCPE and $rCPE_{C186A}$ affected transepithelial resistance (TEER) and 4kDa fluorescent dextran (FD4) transit more quickly than binding-capable, but noncytotoxic rCPE variants, iii) $rCPE_{Y306A/L315A}$ had no effect on TEER or FD4 transit. In mouse intestinal loops challenged with these rCPE species for 2 or 4h, only rCPE was lethal and caused intestinal damage. After 2h of treatment, rCPE was more strongly absorbed into the serum than the other rCPE species but, by 4h, rC-CPE and $rCPE_{D48A}$ had been absorbed similarly as rCPE, while $rCPE_{Y306A/L315A}$ absorption remained modest. This increased rC-CPE and $rCPE_{D48A}$ absorption by 4h was not a general effect on intestinal permeability, since Evans Blue absorption from the intestines was significantly lower in loops treated for 4h with rC-CPE or $rCPE_{D48A}$ vs. rCPE. These results indicate pore formation-induced cytotoxicity causes intestinal histologic damage to facilitate rapid CPE absorption, but cytotoxicity is not required for intestinal CPE absorption. Collectively, these *in vivo* and *in vitro* results support CPE binding to claudin receptors as being sufficient to trigger permeability changes on Caco-2 cell monolayers and a slow but substantial uptake of CPE from the intestinal lumen.


Anaerobe 2022

Sunday, July 31 **Anaerobe Infections**

1415-1620 Session XVII: Clinical Infectious Disease: Anaerobe Infections

⁎—Indicates Presenter

INCREASED VARIETY OF *ACTINOMYCES* INFECTIONS

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Actinomycosis is a long recognized, though rare, endogenous infection caused by a few specific *Actinomyces* species, especially *A. israelii*. These organisms reside on mucosal surfaces, but via trauma, surgical procedures, or foreign bodies they can gain access to deeper tissues. There, aggregates of branching, filamentous bacilli are seen as hard mass-type lesions with a typical histopathologic structure of classical actinomycosis. However, the wide variety of infections caused by these organisms, lack of specific clinical and imaging findings, and their slow growth can make a correct diagnosis challenging. Numerous novel *Actinomyces* species from human sources have been described during the past decades. Recently, reclassifications have been made within the genus; some clinically important species like *A. meyeri*, *A. odontolyticus*, and *A. turicensis* currently belong to a novel genus *Schaalia*, and *A. neuii* to another novel genus *Winkia*. The spectrum of diseases associated with *Actinomyces* / *Schaalia* / *Winkia* species varies and can be located at different body sites. During this brief presentation, I will mainly concentrate on some clinical conditions where these organisms seem to play a relevant role as causative agents. Among these are, for example, *Actinomyces* in medication-related osteonecrosis of the jaw, *S. meyeri* in brain abscesses, *S. turicensis* in skin-related infections, especially below the waistline, and *W. neuii* in infected tissues around various types of prostheses and devices. Since *Actinomyces* / *Schaalia* / *Winkia* organisms are common residents on mucosal surfaces of the human body and appear mainly in polymicrobial infections, they may be regarded as insignificant findings in clinical specimens. Thus, awareness of their potential role as causative agents in a variety of infectious processes is needed to be adequately treated. These organisms are still susceptible to beta-lactams.

DIABETIC WOUND INFECTIONS

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Breach of the skin barrier and subsequent wound healing occur in the context of polymicrobial communities. The microbiome is an important component of the wound environment and is associated with differential healing outcomes. Culture-independent methods to study wound microbiomes have highlighted the abundance and prevalence of mixed anaerobic communities in chronic wounds of various types. Here, we will focus on diabetic foot ulcers, and the role of the microbiome, and especially anaerobic bacteria, in clinical outcomes, including delayed healing and infection. The nature of these communities and their pathogenic mechanisms are understudied despite their prominence and potential as therapeutic targets.

REAL TIME SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN ANAEROBIC BACTERIA

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The development of a real time surveillance platform for monitoring the development of antimicrobial resistance in anaerobic bacteria – ARUMIC.

The prevalence of antimicrobial resistance in anaerobic bacteria is increasing. The consequences of this can be significant. It is therefore crucial that we regularly monitor the development of resistance to raise awareness amongst clinical teams.

The UKARU offers a UK-wide service for the identification and antimicrobial susceptibility testing (AST) of anaerobic clinical isolates. Since 2016, this service has included weekly AST by agar dilution, as recommended by the Clinical Laboratory Standards Institute (CLSI). A standard panel of antimicrobials comprised of Ceftriaxone (CRO), Co-amoxiclav (AUG), Clindamycin (CM), Meropenem (MRP), Metronidazole (MTZ), Piperacillin-Tazobactam (TZP), Penicillin (PEN) and Vancomycin (VA) are included.

The minimum inhibitory concentration (MIC) data is recorded in our Laboratory Information System (LIMS). The data is then curated before being transferred to a microbiology data repository (Datastore). An automated link then generates an MIC distribution that can be visualised via Tableau (interactive data visualisation software). The dashboard allows users to select any combination of organism and antimicrobial and is updated weekly as new MIC data is generated.

The ARUMIC platform allows us to generate real time MIC population distributions for isolates referred from throughout the UK and further afield. It enables continuously updated monitoring of the development of resistance and also assists in the review and development of epidemiological cut-offs (ECOFFs) and species-specific breakpoints, which we are undertaking in collaboration with the European Committee for Antimicrobial Susceptibility Testing (EUCAST).

EUCAST BREAKPOINTS AND METHODS FOR SUSCEPTIBILITY TESTING OF ANAEROBIC BACTERIA

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In the period 2017–2021 the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and its subcommittee on susceptibility testing of anaerobic bacteria, tasked the EDL, and the UKARU with reviewing the reference methodology for agar dilution MIC determination and the development of a standardised disk diffusion method. The task force decided to base both methods on Fastidious Anaerobe Agar (FAA, available from 4 manufacturers). MIC testing was performed on FAA and Brucella Blood Agar (BBA) in parallel.

The species targeted for AST development and for revision of breakpoints by EUCAST were *Bacteroides spp* (n=170)*, Prevotella spp.* (n=49)*, Fusobacterium necrophorum* (n=51)*, Clostridium perfringens* (n=58), and *Cutibacterium acnes* (n=54). For disk diffusion, the inoculum was McF 1.0, temperature 35-37C, and the incubation time a minimum 16 and maximum 20 hours. The correlation between MICs determined on FAA and BBA was excellent except that several of the *F.necrophorum* isolates grew poorly on BBA.

The agents included for the basic development were benzylpenicillin, piperacillin-tazobactam, meropenem, metronidazole and for the Gram-positive organisms also clindamycin and vancomycin.

There was a logical and reproducible relationship between MIC-values and inhibition zone diameters for all agents. However, the importance of the quality of anaerobicity and the need for careful scrutiny of the clindamycin inhibitions zones for microcolonies not to overlook clindamycin resistance were evident.

The development phase was followed by an external clinical laboratory validation trial where 16 laboratories across Europe agreed to test a collection of 35 strains plus quality control (QC) strains. The collective data produced as part of the development were used by EUCAST to review and revise breakpoints for several of the species/agent combinations. Methods, revised breakpoints, and QC criteria are presented in the EUCAST 2022, v 12.0 breakpoint table, available on *www.eucast.org*.

July 28-31

Sunday, July 31 **Model Systems**

1415-1600 Session XVIII: Model Systems to Elucidate the Biology of Anaerobes

*—Indicates Presenter

BIOREACTORS: A VERSATILE TOOL FOR CHARACTERIZING MICROBIAL COMMUNITY DISRUPTION AND RECOVERY

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While disruptions in the gastrointestinal microbiome are associated with several diseases, it can be difficult to separate causation from correlation without tools that allow functional characterization of microbial communities. Continuous flow bioreactors are one tool that have been developed to study responses of microbial communities to perturbations under controlled conditions. To better understand the capacity of high-throughput continuous flow minibioreactor arrays to model dynamics of human fecal communities, we measured community stability and susceptibility to disruption following antibiotic treatment in complex communities cultured from fourteen healthy human fecal donors. We found that differences in microbial composition affected community stability *in vitro*, with enrichment of *Roseburia* and *Phascolarctobacterium* species associated with higher levels of community stability. Community composition also influenced the magnitude of disruption following treatment with different classes of antibiotics, although antibiotics that were broadly (vancomycin, imipenem, clindamycin, ciprofloxacin, and metronidazole) and minimally (sulfamethoxazole, cefaclor, azithromycin, fidaxomicin) disruptive were readily identified. The majority of complex communities cultured *in vitro* were resistant to colonization with *Clostridioides difficile*. Many antibiotics increased susceptibility to *C. difficile* colonization, though there was a wide range of outcomes depending upon community composition and antibiotic used. Somewhat surprisingly, there was little correlation between the overall magnitude of microbiome disruption following antibiotic treatment and susceptibility to *C. difficile* colonization. Ongoing studies are investigating how alterations in diet alter community stability and identifying key similarities and differences between *in vitro* and *in vivo* models of *C. difficile* colonization resistance.

DETERMINANTS OF BIOFILM FORMATION IN THE ANAEROBE GUT SYMBIONT *BACTEROIDES THETAIOTAOMICRON*

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Biofilms are communities of aggregated or surface-attached bacteria exhibiting both beneficial and detrimental specific properties. Despite the medical, environmental, and industrial relevance of biofilms formed by anaerobic bacteria, this widespread lifestyle has mostly been studied in aerobic bacterial species. We investigated biofilm formation in the Gram-negative anaerobe *Bacteroides thetaiotaomicron*, a prominent gut symbiont degrading diet sugars and contributing to gut maturation. Although *B. thetaiotaomicron* capacity to adhere on host or bacterial surfaces and food particles could contribute to gut colonization and homeostasis, little is known on the factors and conditions promoting its biofilm formation.

Using genetic and biochemical approaches, we identify several surface structures involved in *B. thetaiotaomicron* surface adhesion[1]. We also showed that bile is a physiologically relevant gut signal inducing biofilm formation in Bacteroidales. We demonstrated that, although biofilm matrix eDNA usually provides a biofilm-promoting scaffold in many studied bacteria, *B. thetaiotaomicron* bile-dependent biofilm formation requires, on the contrary, an extracellular DNAse degrading matrix eDNA[2]. Our studies provide new perspectives to explore the impact of a gut symbiont biofilm capacity on intestinal microbiota stability and function.

1Béchon N, Mihajlovic J, Vendrell-Fernandez S, Chain F, Langella P, Beloin C, Ghigo JM (**2020**) Capsular polysaccharides cross-regulation modulates *Bacteroides thetaiotaomicron* biofilm formation. ***mBio*** doi: 10.1128/mBio.00729-20

2Béchon N, Mihajlovic J, Lopes A.A I, Sol Vendrell-Fernandez S; Deschamps J, Briandet R, Sismeiro O, Martin-Verstraete I, Dupuy B and Ghigo JM.. Bacteroides thetaiotaomicron uses a widespread extracellular DNase to promote bile-dependent biofilm formation. **bioRxiv** doi:https://doi.org/10.1101/2021.06.04.447082

SYNBIOTIC DELIVERY OF HUMAN MICROBIOTA AND PAIRED FIBER IMPROVES POST-ANTIBIOTIC RESILIENCE IN MICE IN SEX-DEPENDENT WAYS

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Depletion of gut microbiota during antibiotic treatment is known to clear niches that pathogens use to opportunistically dominate the gastrointestinal tract. In turn, dominance of these pathogens (e.g. *Clostridioides difficile, Salmonella enterica,* and *Candida albicans*) generates stable ecological regimes that resist return to pre-antibiotic, healthy states. In this study, we tested whether engraftment efficiency of human microbiota optimized for consumption of a model dietary fiber, sorghum arabinoxylan (SAX), into C57BL/6 mice would be aided by co-delivery of SAX in conventional and antibiotic-treated mice. A human-derived, SAX-consuming consortium of approximately 20 microbial members was selected through sequential passage with SAX as the sole carbon source and was dominated by *Agathobacter rectalis, Bacteroides ovatus,* and *Bifidobacterium longum*. Depending upon their group, mice were gavaged once with the consortium (probiotic) and continuously fed 1% SAX in drinking water (prebiotic), or both (synbiotic). Surprisingly, although we did not find long-term engraftment of any of the members under any condition, the probiotic, prebiotic, and synbiotic treatments differentially altered the mouse gut microbiota in sex-dependent ways; chiefly, succession of the conventional female mice gut microbiota was more susceptible to alteration by all three treatment modalities. Mice of both sexes were treated with poorly-absorbed antibiotics (vancomycin, neomycin, and ertapenem, 1 mg/ml each) for 1 week, which depleted the microbiota. However, synbiotic administration of the consortium + SAX much more rapidly restored microbiome structures resembling pre-treatment microbiota in sex-dependent ways; male mice returned to near-control gut microbiomes three weeks faster and female mice two weeks faster with synbiotic over prebiotic treatment. Our data suggest that even transient microbiota can alter microbiome succession and synbiotic approaches may increase microbiome resilience, thereby reducing opportunities for pathogen colonization.

1230-1330 Poster Session I: Anaerobic Methodology

Posters will be presented in Poster Session I
Friday, July 29 1230-1330.

COMPARATIVE EVALUATION OF MATRIX ASSISTED LASER DESORPTION/ IONIZATION TIME OF FLIGHT MASS SPECTROMETRY (MALDI TOF – MS) ANALYSIS & BIOCHEMICAL CHARACTERIZATION FOR THE IDENTIFICATION OF ANAEROBIC AND MICROAEROPHILIC BACTERIA FROM CLINICAL SPECIMEN

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Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) has found application in various fields of research and diagnosis in the last decade. The unique combination of flexibility, accuracy, automated analysisis of benefit in many fields of biological research made this user friendly test as the choice for species identification. To enumerate the applications of MALDI TOF includes rapid and accurate identification of all bacteria, yeasts and molds, speciation of the Mycobacteria, direct Identification from Positive blood culture, and more recently detection of resistant strains. This technique has not received much popularity in India due the initial cost of the instrument.

Objectives: The present study investigates MALDI TOF analysis for the identification of anaerobic and microaerophilic bacteria from clinical specimen, and also to compare the results with conventional biochemical characterization

Material & Methods: The procedure is so simple either by a Direct spot inoculation of the colony or Extended Direct Transfer after a preparatory protein extraction. A fresh colony of microbe is spot inoculated on plate target slide and readymade matrix (α- cyano 4 hydroxy cinnamic acid) solution is applied to the spot. Sample is submitted to multiple laser shots, matrix absorbs the laser light and vaporizes along with the sample, and the spectra generated from bacterial suspension will be analyzed by the Biotyper software. A score of ≥ 2.0 represents species level, 1.700-1.999 genus level and ≤ 1.7–No identification.

Results: Of the 190 isolates of anaerobes and microaerophils, subjected to MALDI TOF in comparison with culture and conventional techniques, 78% of the isolates were identified upto species level giving concordant results and 22% of strains could be identified only to genus level by conventional techniques. By MALDI many new strains were identified up to species level, especially species of Clostridia, as well as new genera proposed from *Petostreptococcus*.

Conclusion: MALDI TOF is reliable, cost effective, and rapid test for the identification of slow growing microaerophilic and anaerobic bacteria. If facility is available, implementation of MALDI TOF MS as the first step for identification will shorten the turn around time, will reduce the cost in the anaerobic laboratory, and will improve the patient care with better clinical outcome.

IMPACT OF 16S RRNA GENE REGION AND REFERENCE DATABASE ON BACTERIAL COMMUNITY ANALYSES

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Bacterial community profiles in microbiome studies are often generated via PCR amplification of a single variable 16S rRNA gene region. Primer biases, short sequence reads, and database composition can impact classification results, thus selection of a variable region for a specific body site can be a challenge. We evaluated differences in the classification of bacterial species across 6 variable regions, V1-V2, V2-V3, V3-V4, V4-V5, V5-V7 and V7-V9, using human vaginal and urethral samples, as well as mock communities of bacteria assembled from cultivated isolates.

DNA was extracted via the BiOstic Bacteremia DNA Kit. PCR amplicons were generated using the Qiagen QIAseq 16S/ITS Screening Panel and sequencing done via Illumina MiSeq. Reads were de-multiplexed and grouped into variable regions, using the QIAseq 16S/ITS demultiplexer tool within Qiagen CLC Workbench with default settings. The Microbial Genomics Module was used for data QC and taxonomic clustering. Reference databases included Greengenes v13_8, SILVA, and a Fredricks Lab custom urogenital database at 99% clustering.

Species level classification was obtained using the custom urogenital database; hence all comparisons were performed with this database. Variation in bacterial sequence abundance was observed when using different variable regions. Vaginal lactobacilli were best classified using the V1-V2 region (7/7 species vs. 6/7 in V3-V4). Speciation was noted in all regions for common anaerobic bacterial vaginosis associated bacteria such as *Sneathia, Megasphaera, Dialister*, and *Prevotella.* V4-V5 and V7-V9 were unable to speciate abundant *Staphylococcus* species (*S. haemolyticus, S. epidermidis,* and *S. hominis*). *Streptococcus* species were best classified using the V1-V2 region (6/7 abundant species vs. 4/7 in V3-V4).

Different regions of the 16S rRNA gene have differential ability to classify bacterial species within a genus. These findings have implications when selecting a variable region of the 16S rRNA gene for characterizing bacterial communities and may require amplification of more than one region.

DETECTION OF *BAICD* GENE USING QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION IN DOGS: A PROMISING BIOMARKER FOR BILE ACIDS DYSMETABOLISM

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Peptacetobacter (Clostridium) hiranonis is thought to be the main bacterial species responsible for the conversion of primary bile acids (BAs) to secondary BAs through 7α-dehydroxylation in dogs, which is encoded by the *baiCD* gene. This study aimed to assess the correlation between *P. hiranonis*, secondary BAs, and the *baiCD* gene to determine if the *baiCD* gene abundance can be used to predict BAs dysmetabolism. Over 84 days, 133 fecal samples were collected from 24 dogs. 15 of the healthy dogs received metronidazole after the baseline fecal collection. Abundances of *P. hiranonis* and the *baiCD* gene in fecal samples were determined by quantitative PCR. Fecal unconjugated primary and secondary BAs concentrations were measured by gas chromatography mass spectrometry. Spearman's test was used to evaluate their correlation. A $P<0.05$ was set for statistical significance. Similar to *P. hiranonis*, the abundance of the *baiCD* gene was significantly decreased by metronidazole and did not recover completely after 4 weeks in 5 of the 15 dogs who received metronidazole. Abundances of *P. hiranonis* and the *baiCD* gene were positively correlated ($\rho=0.8230$, 95% CI (0.7570, 0.8724), $P<0.0001$). There was a significant correlation between the *baiCD* gene abundance and secondary BAs concentration ($\rho=0.7377$, 95% CI (0.6461, 0.8084), $P<0.0001$) as well as between *P. hiranonis* abundance and secondary BAs concentration ($\rho=0.6658$, 95% CI (0.5555, 0.7532), $P<0.0001$). Since the abundance of the *baiCD* gene, *P. hiranonis*, and the secondary BAs concentrations were positively correlated in dogs' feces, it is possible that the *baiCD* gene abundance may serve as an indicator for BAs conversion. BAs dysmetabolism caused by low secondary BAs concentrations have been associated with chronic gastrointestinal diseases in dogs, dysbiosis and can be observed in dogs following antibiotic treatment. A fast and accessible test for BAs dysmetabolism is needed to screen samples for their BAs converting ability.

COMPARISON OF FOUR STANDARD STOOL COLLECTION METHODS YIELDS MARKED DIFFERENCES IN PHARMACOKINETIC ASSESSMENT FOR MICROBIOME THERAPEUTICS

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Microbiome therapeutics, a promising treatment approach for many indications including *C. difficile* infections and Inflammatory Bowel Diseases, require effective clinical sample collection methods to enable robust pharmacokinetics analysis, but a comparison of the most commonly cited methods has not been conducted to determine which is superior for preservation and detection of product strains. Using strain specific qPCR and 16S sequencing, we tested four sample collection methods with the goal of determining which methods were minimally affected by sample shipment and processing delays leading to long hold times prior to DNA extraction and analysis. Collection strategies tested included two patient sampling approaches using stabilizing buffers and two central lab sampling approaches where whole stool samples were delivered on ice and refrigerated prior to subsampling. Stool samples were collected, bacterial isolates were spiked in, and each sample was homogenized. For patient sampling methods, aliquots for all timepoints were generated at once, stored at room temperature, and moved to the freezer at each timepoint. For central lab sampling methods, stool samples were refrigerated, then subsamples were generated at each timepoint and moved to the freezer. qPCR results showed decreases in the copies per ng of DNA for spiked in strains with central lab sampling approaches, with some strains impacted more than others. Patient sampling methods with preservative buffers yielded the most consistent copies per ng of DNA for all spiked in strains. 16S sequencing showed that community composition for all collection methods deviated similarly from baseline; however, the deviation was more consistent for samples collected in stabilizing buffers. These results point to patient subsampling directly into stabilizing buffer as the preferred stool sampling method for microbiome pharmacokinetic assays.

EVALUATION OF CLINICALLY RELEVANT STOOL COLLECTION METHODS REVEAL BILE ACID DYNAMICS DURING SAMPLE INTAKE AND PROCESSING

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Bile acids are a major focus of interest for many disease areas including *C. difficile* infections, Inflammatory Bowel Diseases, and hepatological indications; however, there are certain real-world constraints on clinical sampling whose effects on bile acid measurement are poorly understood. We investigated bile acid quantification using clinically relevant raw stool sample collection, where stool samples may have to be held during shipment to a central laboratory for intake and freezer storage. Stool donations were collected, and each was homogenized and split into subsamples. One subsample was immediately placed in storage, and primary bile acids were spiked into the second prior to storage to better assess primary bile acid concentrations. At each time point, aliquots were collected and frozen until shipment for quantitative bile acid metabolomics analysis. Large reductions in primary bile acid concentrations were observed with longer storage. Smaller relative changes in secondary bile acids were observed. While an increase in secondary bile acids was observed in samples receiving primary bile acid spikes, higher concentration of secondary bile acids compared to primary bile acids in these stool samples minimized the impact of conversion of primary bile acids on measured secondary bile acid concentrations. These results may help to inform sampling protocols for future clinical studies focused on bile acids.

NEXT GENERATION SEQUENCING ANALYSIS OF A *CLOSTRIDIUM BOTULINUM* OUTBREAK ASSOCIATED WITH HOME CANNED PEAS

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In 2018, just 14 hours after consuming home canned peas, several patients presented to the hospital with symptoms characteristic of botulism. It was suspected that these individuals had consumed food containing botulinum neurotoxins (BoNT), which disrupted communication at neuromuscular junctions resulting in descending paralysis.

Blood and stool specimens were collected and shipped to the New York City Department of Health and Mental Hygiene and the New York State Department of Health (NYSDOH). Real-time PCR (rtPCR) analysis was performed at the NYSDOH to screen for the presence of botulinum neurotoxin (*bont*) genes. In all specimens, *bont* A and B genes were detected. BoNT activity was confirmed using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF/MS); however, it was determined that only active BoNT/A was present in the patient specimens. After epidemiological investigation and environmental sampling, testing was performed on food and tableware collected from the home. The isolates recovered from stool specimens and environmental samples were tested and agreed with all other testing results.

To epidemiologically link and characterize BoNT-producing isolates, a whole genome sequencing pipeline was developed. Enrichment broths were inoculated with isolated colonies and grown at 35°C under anaerobic conditions for 24 hours. Bacterial cells were pelleted and manually extracted prior to preparing libraries using Nextera XT preparation kits. All samples were sequenced using an Illumina MiSeq instrument using 500 cycle v2 reagent kits.

Toxin serotypes for each isolate were assigned by comparing kmers generated from raw reads to unique kmers identified in each *bont* gene. SNP-based analysis was used to compare isolates obtained from outbreak samples. No SNP differences were detected, suggesting that the isolates were indistinguishable. In combination with epidemiological data, we suggest that this analysis provides sufficient evidence to definitively link patient and environmental isolates.

USE OF WHOLE GENOME SEQUENCING (WGS) ANALYSIS TO RETROSPECTIVELY INVESTIGATE *CLOSTRIDIOIDES DIFFICILE* HEALTHCARE ASSOCIATED INFECTIONS

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Clostridioides difficile is an anaerobic, Gram-positive spore-forming rod that is normally found in the gastrointestinal tract. *Clostridioides difficile* infections (CDI) can have symptoms that range from mild diarrhea to severe life-threatening conditions, such as fulminant colitis. The primary virulence factors of *C. difficile* are cytotoxin A and B (*tcdA* and *tcdB)*, as well as two binary toxin genes (*cdtA* and *cdtB*) which contribute to increased pathogenesis of CDI. *C. difficile* is the most prominent cause of nosocomial infectious diarrhea. Understanding sample relatedness is important for infection control and prevention.

For this study, we assessed 50 isolates from 6 retrospective outbreak investigations across NYS. Isolates were identified as *C. difficile* using MADLI-TOF MS and real-time PCR was used to detect the DNA of toxin genes, *tcdA, tcdB, cdtA,* and *cdtB.* Pyrosequencing was performed on toxin positive samples to determine if there was a 1bp, 18bp or 39bp deletion present in the *tcdC* gene. Additionally, Pulsed-Field Gel Electrophoresis (PFGE) and WGS were utilized to determine genetic relatedness of all isolates. WGS analysis was performed using three methods: an in-house developed pipeline that assesses isolate relatedness through Single Nucleotide Polymorphism (SNP) and insertion/deletion (indel) analysis, an in-house developed core-genome based allele pipeline, and the NCBI Pathogen Detection Browser. WGS provided greater resolution of the investigation clusters than PFGE. In addition, WGS analysis performed with the in-house developed pipeline demonstrated greater resolution of clusters than the two allele-based analysis methods. WGS represents an efficient and cost-effective method to characterize *C. difficile*, determine relatedness, and improve our understanding of healthcare associated outbreaks of *C. difficile.* Ultimately, this will enhance of the ability of healthcare institutions to implement infection control measures and prevent CDI.

RECOMMENDATION FOR UPDATING CLSI METRONIDAZOLE BREAKPOINTS FOR ANAEROBES

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Metronidazole susceptibility breakpoints for anaerobic organisms vary between Clinical and Laboratory Standards Institute (CLSI) (all anaerobes: susceptible [S] ≤8 mcg/mL; resistant [R] >16 mcg/mL) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (v11.0 gram-negative and gram-positive anaerobic organisms: S ≤4 mg/dL; *Clostridioides difficile* S ≤2 mg/dL). We reviewed metronidazole susceptibility minimal inhibitory concentrations (MICs) of a large population of gram-positive and gram-negative anaerobes and interpreted the MICs according to the two different sets of breakpoints. From May 2020 to October 2021, metronidazole MIC testing by was performed agar dilution per CLSI M11-A8 on 8699 clinical anaerobic isolates recovered by or sent to our laboratory for testing.

At the time of testing, 6294/8699 (72.4%) isolates tested at an MIC of ≤4 mcg/mL, and 6448/8699 (74.1%) tested at an MIC of ≤8 mcg/mL. Per EUCAST, 2374/8699 (27.3%) were R, while 2111/8699 (24.3%) were R per CLSI. When organisms with putative intrinsic resistance to metronidazole (non-sporeforming anaerobic Gram-positive bacilli) were excluded from the data set, 5834/6027 (96.7%) and 166/6027 (2.6%) were S and R per EUCAST, respectively, while 5937/6027 (98.5%) and 44/6027 (0.7%) were S and R per CLSI, respectively. Non-sporeforming anaerobic Gram-positive bacilli represented 30.7% of the organisms tested (2067/8699), but 98% of organisms reported R per CLSI, and 82.5% of organisms reported intermediate per CLSI. EUCAST issued revised breakpoints Jan 2022 (v12.0 *Bacteroides, Prevotella,* and *Clostridium perfringens* at ≤4 mg/dL, *Clostridioides difficile* at ≤2 mg/dL); for this subset of organisms, 3064/3104 (98.7%) and 40/3104 (1.3%) would be S and R per EUCAST, respectively, while 3095/3104 (99.7%) and 2/3104 (0.06%) were S and R per CLSI, respectively.

In light of increasing clinical concern regarding the development of resistance to metronidazole in anaerobic organisms, data suggest that reducing the clinical breakpoint for susceptibility to ≤4mcg/mL would provide increased faculty for monitoring this emerging trend.

July 28-31

Friday, July 29 — Anaerobic Pathogenesis

1230-1330 Poster Session I: **Anaerobic Pathogenesis**

Posters will be presented in Poster Session I
Friday, July 29 1230-1330.

TRANSCRIPTOME OF EPIBIONT *SACCHARIBACTERIA* TM7X DURING ESTABLISHMENT OF SYMBIOSIS

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Despite their broad presence, very little is known about how the bacteria of the recently defined Candidate Phylum Radiation establish and maintain themselves as obligate epibionts on bacterial hosts. We conducted the first transcriptome analysis of a radiation member, *Saccharibacteria Nanosynbacter lyticus* type strain TM7x, during the establishment of symbiosis on its host, the anaerobe *Schaalia odontolyticus* strain XH001. Infected host with TM7x was sampled during their initial interaction and after attaining stable symbiosis. Naive host controls following the same culturing were sampled at the same time points. Voom/limma analysis with a FDR cutoff of 0.05 was applied to three host comparisons, initial interaction versus naive, stable symbiosis versus naive, and symbiosis versus initial, as well as symbiosis versus initial for TM7x. Significant differences in RNA expression were seen between infected host and control during the initial interaction and stable symbiosis, 7% and 12% of the genome respectively. Extensive differences were seen between symbiosis and the initial encounter for both host, 30%, and TM7x, 60%. The changes in cellular functions and pathways have given us new insight into the poorly understood process of establishing a stable bacteria/ bacteria epibiont/ host interaction.

EFFECTS OF *SARGASSUM FUSIFORME* ON RUMEN MICROBIOTA, FERMENTATION CHARACTERISTICS, AND METHANE PRODUCTION USING *IN VITRO*

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Background: The rumen microbiota is recognized as major regulator of ruminant physiology and health. In this study, we investigated the effect of *Sargassum fuisforme* (*S. fusiforme*, species of brown seaweed) extracts on the rumen microbiota, fermentation characteristics, and methane production *in vitro* for developing feed additives.

Methods: Anaerobic rumen fluid was collected from two non-lactating cannulated Hanwoo cows, and *in vitro* batch culture was performed up to 48 h incubation. Concentration of CH4 in the gas samples were determined by a gas chromatography (Shimadzu, GC-2010 PLUS, Japan) equipped with HP-PLOT Q capillary column (I.D. 0.53 mm, L.30 m) and flame ionization detector (FID). Paired-end sequencing (targeting V3-V4 region) was carried out at Macrogen (Seoul, Korea) on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). All the data was analyzed by QIIME2 (version 2021.02) software and operational taxonomic units (OTUs) were assigned to the pre-trained Greengenes (13_8 version).

Results: Compared with control (CON, without *S. fusiforme*), addition of *S. fusiforme* was significantly ($P<0.05$) increased total volatile fatty acid and molar proportion of propionate. However, molar proportion of butyrate and ammonia nitrogen concentration was significantly ($P=0.06$ and $P<0.01$, respectively) decreased by the addition of *S. fusiforme* than CON. Compared with CON, methane (CH4) production was significantly ($P<0.05$) decreased about 23% by the addition of *S. fusiforme*. The similar responses of *S. fusiforme* treatment was seen not only *in vitro* dry matter digestibility but also rumen microbiota composition compared with the CON. At the phylum level, Euryarchaeota, Firmicutes, and Proteobacteria were higher at *S. fusiforme* than CON, whereas Bacteroidetes, Spirochaetes, and Lentisphaerae were lower at *S. fuiforme* than CON. At the genus level, relative abundance of Prevotella, Selenomonas, Paludibacter were lower at *S. fusiforme* than CON. However, relative abundance of *Succiniclasticum, Christensenella, Methanobrevibacter* were higher at *S. fusiforme* than CON. There may be several mechanisms involved in the positive responses of the addition of *S. fusiforme* on the rumen fermentation and ecosystem.

Conclusion: These results suggest that the addition of *S. fusiforme* can modulate ruminal fermentation to alter volatile fatty acids concentration and reduce CH4 production by altering rumen microbiota.

EFFECTS OF THE SAME *NIM* GENE-INSERTION SEQUENCE CONFIGURATIONS ON THE EXPRESSION OF THE *NIM* GENES AND METRONIDAZOLE RESISTANCE OF *BACTEROIDES FRAGILIS* STRAINS

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For metronidazole resistance of *Bacteroides spp.,* the best-characterized resistance mechanism is that which is mediated by *nim* genes. However, there are some issues that are not supporting this view, e.g. some strains carry a *nim* gene but are not metronidazole resistant and sometimes the nim expression does not correlate with metronidazole resistance. Previously, we reported some *nimB* and *nimE*-postive *B. fragilis* strains that had gene-specific insertion sequence (IS) elements that are thought to regulate the expression of these genes. However, despite the same genetic constitution, these strains differed in the levels of metronidazole resistance.

Our aim was to investigate this odd relationship. We recorded metronidazole MICs by Etests, isolated total RNA and by gene-specific primers we conducted qRT-PCR to detect the expression of *nim* (*nimB* 6, *nimE* 10 *B. fragilis* strains) and *cfiA* genes. Metronidazole resistance scattered through a wide range for our test strains, but the expression of the *nim* corresponded to the actual IS content (*nimA*-IS*1168*, *nimE*-IS*Bf6*). *CfiA* expression also depended on the activating IS elements. The *nimE* expression levels were higher than those of *nimB* genes since the *nimE* genes were located on multicopy plasmids.

Therefore, we can conclude that IS elements regulate the expression of *nim* genes, but to explain the role of the *nim* genes in metronidazole resistance we propose that some rate limiting steps also influence the actual metronidazole MICs

INVESTIGATIONS ON THE CLONALITY OF THE NOVEL *CRXA* METALLO-β-LACATAMASE GENE-CARRYING *BACTEROIDES XYLANISOLVENS* STRAINS

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In our laboratory, a novel metallo-β-lactamase gene was described from an imipenem-resistant *B. xylanisolvens* strain. Furthermore, we detected some 7 additional *B. xylanisolvens* strains that were also *crxA*-positive. *CrxA* had some commonalities with *cfiA,* insertion sequence (IS) activation, a specific genetic element with a GCN5-like acetylase toxin gene on it and a similar prevalence (16.7%) among the strains of this species, above the metallo-β-lactamase nature.

We were interested in if the *crxA*-positive *B. xylanisolvens* strains are also genetically related as the *cfiA*-positive *B. fragilis* strains. 22 *B. xylanisolvens* strains were included of which 8 were *crxA*-positive. We used conventional and inverse PCR to detect the proposed genetic element, enterobacterial repetitive intragenic consensus PCR typing and MALDI-TOF MS.

Of the 8 cases examined 5 had the same genetic element (ca. 2.5 kb) out of which 3 were inserted to the same chromosomal regions of the *B. xylanisolvens* chromosomes which would have permitted clonality. However, the ERIC PCR typing did not confirm this, all the *crxA*-positive strains displayed different ERIC PCR patterns. The MALDI-TOF spectra were uniform not allowing enough resolution to differentiate between the *crxA*-positive and negative strains.

In conclusion, we can say that there may be different genetic elements that harbor *crxA* of which one is integrating to a specific chromosomal locus and *crxA* does not show clonality which allows that it can be mobile.

July 28-31

Friday, July 29 — Antimicrobials

1230-1330 Poster Session I: Antimicrobials

Posters will be presented in Poster Session I
Friday, July 29 1230-1330.

VE707, A LIVE BIOTHERAPEUTIC PRODUCT FOR INFECTION PREVENTION OF MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA

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Infections with multidrug-resistant organisms (MDRO) are increasing at an alarming rate in hospitals worldwide. Due to the rapidly growing threat of antibiotic resistance, there is an urgent unmet need for novel therapies to tackle MDRO infections. Intestinal MDRO colonization, resulting from microbiota disruption, frequently precedes infection with the colonizing organism. As such, decolonization strategies such as non-absorbable antibiotics and fecal microbiota transplantation (FMT) have shown efficacy at preventing infection following decolonization. Despite the success of FMT at resolving intestinal MDRO colonization and dysbiosis without leading to resistance, FMT efficacy is variable and its safety profile questionable. This highlights the need for a uniform, well-characterized microbiome-based product with robust efficacy that can be produced and administered in a standardized manner.

Vedanta is developing VE707, a defined live biotherapeutic product (LBP) consisting of beneficial gut bacteria to reduce intestinal carriage of carbapenem-resistant and ESBL-producing *Klebsiella pneumoniae* (Kpn) and *Escherichia coli* (Eco), restore a healthy microbiota, and prevent infection and colonization recurrence. Using a top-down approach, we first characterized fecal material from healthy individuals for their ability to suppress Kpn and Eco and identified a donor enriched for activity against both pathogens. Next, we used a series of *in vitro*, *in silico*, and *in vivo* tools to assemble bacterial strains from this donor into LBPs and evaluated their ability to decolonize Kpn and Eco in a mouse co-colonization model. Of 70 LBPs screened, VE707 showed the greatest decolonization efficacy as demonstrated by a ≥3-log reduction in Kpn and Eco fecal levels. Furthermore, VE707 was active against several MDR Kpn and Eco clinical isolates and prevented colonization recurrence in decolonized mice. Our results show that VE707, a defined bacterial consortium with pathogen-antagonistic properties, was successful at decolonizing Kpn and Eco, and it is currently in manufacturing phase.

ANTIMICROBIAL ACTIVITIES OF BACTERIAL PROBIOTIC CULTURES AGAINST LIVER ABSCESS-CAUSING PATHOGENS IN BEEF CATTLE

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Liver abscesses are bacterial pyogenic infections that occur in beef cattle fed high-grain, low-roughage diets. The causative agents include two subspecies of *Fusobacterium necrophorum* (*necrophorum* and *funduliforme*), *Trueperella pyogenes*, and *Salmonella enterica*. These pathogens originate from the rumen or hindgut of cattle and enter the portal circulation to reach the liver to cause abscesses. Tylosin, a macrolide antibiotic, is supplemented in the feed to reduce liver abscesses. However, the concern for the emergence of antimicrobial resistance has necessitated the need to find alternatives to replace tylosin. Among antibiotic alternatives, probiotics have gained wide acceptance in the cattle industry, because they are considered natural and generally recognized as safe products. We tested antimicrobial activities of culture supernatants of certain bacterial probiotic cultures against liver abscess-causing bacterial pathogens. Probiotic cultures tested included *Lactobacillus helveticus, L. rhamnosus, L. acidophilus, L. buchneri*, *Pediococcus acidilactici*, *Pediococcus pentosaceus, Bacillus pumilus,* and *Bacillus subtilis*. Probiotic bacterial species were cultured in appropriate growth media, centrifuged, filter sterilized, and stored at -20°C. Liver abscess-causing bacterial species were cultured in Muller-Hinton broth (for *S. enterica* and *T. pyogenes)* and anaerobic Brain-Heart infusion broth (for *Fusobacterium*), with and without probiotic culture supernatant. Bacterial growth was measured in a spectrophotometer and spread-plated onto blood agar plates at 2, 4, 6, and 8 hours of incubation to measure bacterial concentration. Only the supernatant of *L. helveticus* reduced the growth of both *Fusobacterium* subspecies, *T. pyogenes* and *S. enterica*. Addition of *L. helveticus* supernatant to *in vitro* fermentations containing ruminal fluid, buffer, and substrates (glucose, lactic acid, or ground cattle feed) exhibited reduction in spiked culture of *F. necrophorum*. Probiotic cultures, such as *L. helveticus* may have the potential to be used as a feed supplement to control liver abscesses.

ANTIMICROBIAL PHOTODYNAMIC THERAPY – IS 5 AMINOLEVULINIC ACID THE MAIN EFFECTIVE AGENT?

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Bacterial colonization of ulcers can interfere with the healing process. 5-Aminolevulinic acid (5-ALA) is utilized as a photosensitizer for performing a photodynamic therapy (PDT). We studied if 5-ALA can effectively increase bactericidal action of PDT against selected strains of *Clostridioides difficile, Clostridium perfringens,* and *Candida albicans.*

Strains of *C. difficile* (ATCC BAA 1804 and ATCC BAA 1870), *C. perfringens* (ATCC 19408), *C. albicans* (ATCC 14053 and ATCC 18804) were inoculated on Columbia Blood agar (CBA) and Sabouraud agar (SA), respectively, incubated for 24 - 48 h at 37°C under anaerobic/aerobic condition. 2 McFarland (MF) scale suspensions in PBS were prepared and 300 µL of each solution was used. From *C. albicans strains* cultured on SA suspensions of 0,5 MF were prepared in PBS.

Stock solutions of 5-ALA were prepared in deionized water, further dilutions were made to obtain concentrations, commonly used for actinic keratosis treatment: 1000 µM and 20%.

The laser light source was Viofor S-PTD (Med&Life, Poland) device: wavelength 670 nm, power 10 J/cm2, time of exposition - 3 minutes. Six controls and study groups were studied.

The PDT alone inhibited growth of *C. difficile, C. perfringens,* and *C. albicans,* yet inhibition reached 20%, no significant inhibition of examined strains by used concentrations of 5-ALA was noted. The PDT procedure with both concentrations of 5-ALA was significantly inhibiting for all of studied strains with CFU reduction by 90% (p=0,00001). Photodynamic antibacterial therapy is one alternative method to overcome antibiotic-resistant strains of bacteria.

This study was partially financed by Medical University of Silesia Grants PCN-1-126/K/O/I and PCN-2-065/N/0/O.

METRONIDAZOLE RESISTANCE IN ANAEROBIC INTRA-ABDOMINAL INFECTIONS: A GROWING MENACE?

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Background: Intra-abdominal infections (IAI) are comprised of diverse clinical conditions, ranging from uncomplicated appendicitis to life-threatening situations like florid fecal peritonitis which constitute the primary diagnosis in 8% of hospitalizations. Gut microbes are common etiological agents in intra-abdominal infections, and *Bacteroides fragilis* group are the most commonly recognized anaerobic pathogens. In this study, we aim to characterize anaerobic isolates from IAIs and analyse their antimicrobial susceptibility against metronidazole.

Methods: Present study was conducted between January and December 2017 in the Microbiology laboratory attached to a tertiary care hospital. Specimens, such as pus aspirates, soft tissue, and peritoneal fluid from diverse IAIs were included. The specimens were inoculated on anaerobic blood agar, neomycin blood agar and were incubated in anaerobic workstation (Don Whitley's anaerobic workstation, A35, Yorkshire, UK). Anaerobes were identified by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (Vitek MS, bioMerieux Inc., France). β-lactamase production was detected using nitrocephin disks (BD BBL Cefinase, Becton Dickinson and Co, Sparks, USA) . E test method (bioMerieux Inc., Marcy L'Etoile, France) was used to determine the antimicrobial susceptibility of metronidazole. The results were interpreted following Clinical Laboratory Standards Institute (CLSI) guidelines

Results: A total of 58 specimens from IAIs were analysed, of which anaerobic growth was observed in 39.7% (23) of specimens. Intra-abdominal abscess was the most commonly presenting clinical condition. Anaerobic Gram negative bacilli (71.4%) were the common isolates, of which *B. fragilis* was the most frequent anaerobe isolated (45.7%) followed by anaerobic Gram positive cocci (22.9%). Metronidazole resistance was observed among 3 isolates (13%) of which, two were *Prevotella* spp. (MIC 32 µg/mL & 256 µg/mL) and one *B. fragilis* (MIC 32 µg/mL). β-lactamase activity was detected in all *B. fragilis* group isolates.

Conclusion: Increasing trend in metronidazole resistance among anaerobic pathogens may jeopardize its role as empirical therapeutic choice in future, particularly in developing countries which lack the facilities of routine anaerobic susceptibility testing. It is time to look into metronidazole resistance carriage among intestinal anaerobic flora. Detection of probable horizontal transmission of resistance across species is the need of the hour which can be directly benefit patient management.

THE GENOME AND EVOLUTIONARY ANALYSIS OF MULTI-DRUG RESISTANT *BACTEROIDES FRAGILIS* ISOLATES FROM INDIA

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A statement of the purpose: Very few MDR genomes of *B. fragilis* are available limiting our knowledge on antimicrobial resistance (AMR) evolution. Thus, the current study focuses on the genome and evolutionary analyses of MDR *B. fragilis* isolates from India.

Methods: The MDR *B. fragilis* strains (3/100) resistant to clindamycin, metronidazole, cefoxitin and piperacillin-tazobactam were subjected to whole genome sequencing via Illumina technology. The raw reads were filtered and trimmed using PRINSEQ followed by de novo assembly using CLC Genomics Workbench. The assembled genome was annotated using RAST. For functional classifications, COG and KEGG databases were used. The AMR genes were predicted using CARD and ResFinder. Towards evolutionary exploration, pan-genome, horizontal gene transfer (HGT), and selective pressure analyses were performed using BPGA, HGTector, and KaKs_Calculator, respectively.

Results: The MDR *B. fragilis* isolates (n=3) showed open pan-genome. The estimate was shown after using 30 random samples of the total 175 *B. fragilis* genomes available. The COG classification showed that metabolic genes were the most predominant in the core genome. The *in-silico* HGT analysis revealed that in *B. fragilis* strains, approximately 7-8% of the total genes were predicted to be acquired horizontally. Among AMR genes, *nimE* and *cfiA* genes were predicted to be acquired via HGT. The selection pressure analysis showed that *nimE* and *ermF* genes were under positive selection; in contrast, *cfxA, cfiA, tetQ,* RND efflux system, inner membrane transporters were under negative selection pressure.

Conclusion: *B. fragilis* showed an open pan-genome, with high genomic plasticity indicating continuous evolution and the ability to obtain new genes via HGT. The genes conferring resistance to clindamycin (most resistant drug) and metronidazole (the mainstay drug of anaerobic infections) were seen under strong positive selection pressure; suggesting, the genes may evolve under considerable selection pressure leading to the fixation of resistance determinants. Thus the judicious use of anaerobic drugs is the need of the hour.

DEVELOPMENT OF CARBAPENEM RESISTANCE IN *BACTEROIDES FRAGILIS* BACTEREMIA ASSOCIATED WITH INTRA-ABDOMINAL ABSCESS UNDER ANTIMICROBIAL PRESSURE

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Multi-drug resistant (MDR) Bacteroides are still rare but exist in clinical settings and tend to complicate treatment. We isolated MDR *Bacteroides fragilis* from the bloodstream and intra-abdominal abscesses of a patient who had undergone a series of abdominal operations under antimicrobial pressure.

The present case involves a 52-year-old man with known ulcerative colitis since 2011. Complex medical history included hypertension, diabetes mellitus, hypertrophic cardiomyopathy and hypothyroidism. In 2016, the patient failed to show a complete response to medical therapy and subtotal colectomy with terminal ileostomy with mucous fistula was performed by general surgery. Six months later, patient underwent restorative colectomy with ileal pouch-anal anastomosis. On post-operative day 20, empiric treatment with ampicillin-sulbactam was initiated due to the development of intra-abdominal abscess. Abdominal fluid culture isolates were identified as *Enterococcus faecalis*, extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, *Peptoniphilus lacrimalis* and *B. fragilis*. Ciprofloxacin was added to the patient's antimicrobial regimen, percutaneous drainage was performed again after five days. Culture from additional intra-abdominal fluid collection also grew ESBL-producing *E. coli, B. fragilis* and *Peptostreptococcus anaerobius*. Ertapenem was added to the patient's antimicrobial regimen. He was discharged from the hospital on the third day of ertapenem treatment due to his good clinical condition.

Two months later, patient presented at our emergency department with fever. Blood cultures were obtained, and *B. fragilis* was detected in one set of blood culture. *B. fragilis* isolates from three samples showed the same spectrum characterized by Repetitive Extragenic Palindromic-PCR performed with the BOX A1R primer. These three isolates demonstrated resistance by epsilometer test (E-test) to amoxicillin-clavulanic acid, metronidazole, clindamycin, chloramphenicol and moxifloxacin. On the other hand, the MIC value of meropenem gradually increased from the higher MIC value to the resistance. (3, 4, 8 mg/L, respectively) cfiA gene encoding the carbapenemase was identified in three isolates.

In order to prevent the danger and control the resistance, it is necessary to determine the resistance profile of the bacteria to antibiotics and the resistance genes at regular intervals.

EFFECT OF METRONIDAZOLE ON VAGINAL BACTERIA ASSOCIATED WITH RISK OF HIV ACQUISITION

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Background: Women with bacterial vaginosis (BV) have complex vaginal bacterial communities. Several bacteria in these communities are associated with risk of HIV acquisition, but their susceptibility to antibiotics is poorly understood. We sought to characterize how vaginal concentration of select bacterial taxa associated with increased HIV risk change following antibiotic treatment for BV, in order to identify effective interventions for their eradication.

Methods: Vaginal samples were collected from 36 women enrolled in a longitudinal study of BV. Swabs were collected daily for two weeks following BV diagnosis, during which antibiotic treatment with metronidazole was administered orally or as a vaginally applied gel for 5-7 days. DNA was extracted and subjected to a suite of taxon-specific 16S rRNA gene quantitative PCR (qPCR) assays to monitor changes in concentration of taxa associated with HIV risk. A culture-based antimicrobial test was developed to confirm the presence of antibiotics in vaginal swab samples.

Results: Bacterial DNA concentration decreased over the duration of antibiotic administration for all thirteen bacterial taxa tested. Comparison of bacterial 16S rRNA gene copy numbers from samples taken before administration of antibiotics to samples taken on the last day of assay-confirmed antibiotic presence showed a 2.3-4.5 log-fold decrease across all taxa. In many instances, qPCR copy number was reduced to the assay's limit of detection, suggesting eradication of bacteria. Mean time to clearance also varied greatly between taxa (1.2-8.6 days), with bacteria associated with HIV risk (e.g. *Gemella asaccharolytica*, *Sneathia* spp., *Eggerthella*-like sp.) often taking >7 days to suppress.

Conclusions: Administration of metronidazole reduces quantities of vaginal bacterial taxa associated with increased risk of HIV acquisition. Eradication of high-risk vaginal bacteria using metronidazole is one promising avenue to explore for reducing women's risk for HIV acquisition. However, a 5-7 day treatment course may not be sufficient to suppress all vaginal bacteria associated with increased risk of HIV acquisition.

Friday, July 29 — Fusobacteria

1230-1330 Poster Session I: Fusobacteria

Posters will be presented in Poster Session I
Friday, July 29 1230-1330.

Friday, July 29 — Fusobacteria

1230-1330 Poster Session I: Fusobacteria, *continued*

PI-29 Association of *Fusobacterium* Species with Colon Cancer and Translation to Mouse Models 142

Queen, J.; Drewes, J.L.; White, J.R.; Zhuang, Y.; McMann, M.; Wu, S.; Wanyiri, J.; Vadivelu, J.; Iyadorai, A.; Roslani, A.C.; Sears, C.L.*

PI-30 Study of *Fusobacterium nucleatum* Type II Fatty Acid Synthase System Using Molecular and Chemical Genetics 143

Rutherford, J.T.; Dureja, C.; Norseeda, K.; Sun, D.; Hevener, K.E.; Hurdle, J.G.*

Posters will be presented in Poster Session I
Friday, July 29 1230-1330.

OUTER MEMBRANE VESICLES OF *FUSOBACTERIUM NECROPHORUM*

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Fusobacterium necrophorum, a Gram-negative obligate anaerobe, is the etiological agent of liver abscesses and necrotic infections in cattle. Outer membrane vesicles (OMVs) shed by pathogenic bacteria contain periplasmic contents including toxins, virulence factors, lipoproteins, and others. The immunogenicity and pathogenicity of OMVs released by *F. necrophorum* have not been studied previously. We investigated the virulence factors of *F. necrophorum* released in OMVs through proteomics and lipid profile study. Understanding the immunogenicity and pathogenicity of these vesicles could help identify the vaccine potential of OMVs against fusobacterial infections. In the current study, the OMVs from late log phase *F. necrophorum*-spent culture were concentrated using ultrafiltration and ultracentrifugation. The extracted OMVs were purified by OptiPrep density gradient method and were analyzed by electron microscopy (EM). OMV preps were then subjected to proteomics and lipid profile analysis. The proteomics study identified major virulence factors such as leukotoxins, adhesins (43kDa-FomA, 22kDa-OmpA, 17kDa-OmpH, FadA, and 66kDa-CSP) and other autotransporter domain containing proteins in higher abundance than in vegetative bacteria. Some of the identified proteins were confirmed through western blot analysis using corresponding antibodies. Similarly, lipid profile study revealed the presence of phospholipids, such as phosphatidylethanolamine (PE), phosphatidylcholine-sphingomyelin (PC-SM), phosphatidylglycerol (PG) along with acetyl carnitine (AC), as major lipid components of OMVs. We performed a differential study of OMV production in iron deficient versus iron rich conditions. This study showed relative increase in the yield of small-sized OMVs with high protein content in iron limiting conditions. We plan to investigate the immunogenic response in a mouse challenge model and the pathogenicity effect through co-culture and cytotoxicity assays in our next phase of study. To sum up, insights of OMVs contents could help develop potent vaccines against fusobacterial infections.

HYDROGEN SULFIDE BIOSYNTHETIC ENZYMES ARE REQUIRED FOR *FUSOBACTERIUM NUCLEATUM* FITNESS, ANTIBIOTIC SENSITIVITY, AND VIRULENCE

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Fusobacterium nucleatum is one of major oral producers of hydrogen sulfide (H_2S) – a volatile sulfur compound significantly responsible for halitosis. However, genetic determinants of H_2S production and its association with fusobacterial fitness and virulence are not well understood. We show here that *F. nucleatum* encodes four enzymes CysK1, CysK2, HlY, and MegL, known to metabolize L-cysteine to H_2S, with the L-cysteine desulfhydrase CysK1 previously shown to produce the most H_2S *in vitro*. Expression analysis revealed that *cysK1* and *megL* are highly expressed during exponential growth, whereas expression of the other genes remains low. Deletion of *cysK1* is lethal, unless lanthionine is provided. Interestingly, this conditioned *cysK1* mutant does not reduce H_2S production, neither do *cysK2* and *hly* mutants. In contrast, a *megL* mutant displays a drastic reduction of H_2S production, while ectopic expression of *megL* rescues this defect. When these mutant cells are exposed to various antibiotics for a short time, the *megL*, *cysK1* and *cysK2* mutants become sensitive to ampicillin, while the last two are also sensitive to nalidixic acid, as compared to the parent strain. Intriguingly, with prolonged exposure to these antibiotics, only the *megL* mutant exhibits resistance to kanamycin and increased susceptibility to nalidixic acid, unlike the parent strain. Importantly, in a mouse model of preterm birth the *megL* mutant is significantly attenuated in virulence, for its spreading to and colonization of placenta and fetus is markedly reduced. Evidently, MegL is a major H_2S producer *in vivo* that significantly contribute to bacterial fitness, antibiotic susceptibility, and virulence.

NEW INSIGHTS INTO *FUSOBACTERIUM NUCLEATUM* TRANSFORMATION: IMPLICATIONS FOR THE DEVELOPMENT OF A BROADLY APPLICABLE FUSOBACTERIAL GENETIC SYSTEM

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Fusobacterium nucleatum is a Gram-negative obligate anaerobe and a typical member of the human oral microbiome. *F. nucleatum* is strongly associated with the development of multiple mucosal inflammatory diseases and various malignant tumors. Studies of *F. nucleatum* genetics are notoriously challenging due the genetic intractability of the vast majority of strains. In this study, we isolated a diverse collection of multiple *F. nucleatum* subspecies directly from clinical odontogenic abscess and plaque specimens. This collection, along with an ATCC strain was subsequently investigated to identify and characterize strains amenable for genetic manipulation using natural and/or artificial transformation approaches. Our results reveal unique features of *F. nucleatum* genetics that may be exploitable to greatly improve the genetic tractability of this species, especially with low-passage clinical isolates.

COMPARING THE ANTIMICROBIAL SUSCEPTIBILITY RESULTS OF CLINICALLY RELEVANT *FUSOBACTERIUM SPECIES* DETERMINED BY AGAR DİLUTİON AND THE TENTATIVE EUCAST DİSK DIFFUSION METHOD

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The aim was to define antimicrobial susceptibility of *Fusobacterium* clinical isolates using agar dilution methods with different media and the tentative EUCAST disk diffusion method, and to determine the compatibility of agar dilution tests on different media

A total of 39 non-duplicate *Fusobacterium* strains, isolated from various clinical specimens at tertiary care hospital in Istanbul, Turkey – between January 2015 and April 2021 – were identified using MALDI-TOF MS. MICs of ampicillin, ampicillin-sulbactam, clindamycin, meropenem, metronidazole, and moxifloxacin were determined by dilution method using sheep blood supplemented Brucella agar (BA) and horse blood supplemented Fastidius Anaerobic Agar (FAA).The antimicrobial susceptibility of isolates were also tested using tentative EUCAST disk diffusion method on FAA.

Four different *Fusobacterium* species were identified, *F. necrophorum* (n=18, 46%), *F. nucleatum* (n=16, 41%), *F. periodonticum* (n=3, 8%) and *F. varium* (n=2, 5%). In total 30.8% (n=12) of the patients had malignancies, and *F. nucleatum* was the most prevalent species in the respective patients.

Five of the 39 isolates didn't grow on BA, despite the consecutive test repetitions. While, on FAA, all strains were able to grow on the media at first performance, both with agar dilution and disc diffusıon methods. These 5 isolates, except for a single strain with an ampicillin MIC of 1 µg/ml, were susceptible to all antibiotics tested on FAA, according to EUCAST 2021 criteria.

According to the agar dilution results, the isolates were 91% sensitive to ampicillin, 97% to clindamycin, and 91% to moxifloxacin, and no resistance was found to the other tested antibiotics on both media. MIC values on FAA and BA were found to be compatible for clindamycin, meropenem and metronidazole, whereas essential and categorical agreement for ampicilin, ampicilin-sulbactam, and moxifloxacin were not high enough.

According to the disk diffusion results, 20 hours of incubation was found to be sufficient for evaluation.

In conclusion detection of to ampisilin, clindamycin, and moxifloxacin non-susceptible isolates necessitates the careful use of these antibiotics in terms of treatment failure in *Fusobacterium* infections. It is pleasing to find out a good correlation between the dilution methods on FAA and BA for clindamycin, meropenem and metronidazole.

COMPLICATIONS IN PHARYNGOTONSILLITIS PATIENTS INVESTIGATED FOR BETA-HEMOLYTIC *STREPTOCOCCI* AND *FUSOBACTERIUM NECROPHORUM*

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Background: European guidelines on pharyngotonsillitis management focus on identification and treatment of Group A *Streptococci* (GAS) with the use of rapid antigen detection tests (RADT) in patients with Centor score of 3-4. Yet, many RADT-negative patients are treated as well as further evaluated for Group C or G *Streptococci* (GCS/GGS) and *F. necrophorum.* The study purpose was to investigate associations of a finding of *F. necrophorum* or beta-hemolytic *Streptococci* with complications in pharyngotonsillitis patients.

Methods: Patients diagnosed with pharyngotonsillitis and tested for *F. necrophorum* (PCR) and beta-hemolytic *Streptococci* (culture) were enrolled from June 2013-December 2020 in the Skåne Region, Sweden. Patients with prior complications or antibiotics (30 days) were excluded. Data was retrieved from registries and electronic charts. The primary outcome was a composite score of complications within 30 days, defined as peritonsillar or pharyngeal abscess, otitis, sinusitis, sepsis or septic complications, chronic or recurrent tonsillitis (after 15-30 days) or hospitalization. Crude and adjusted logistic regression analyses were performed and odds ratios (OR) presented.

Results: In total, 4004 visits were included. *F. necrophorum* was identified in 1144 (29%), GAS (by RADT or culture) in 431 (11%), GCS in 299 (7%) and GGS in 209 (5%). 2145 (54%) had both negative PCR and culture. Complications were seen in 33% of patients with GAS, 30% with *F. necrophorum*, 22% with GCS, 20% among negative and 13% with GGS. *F. necrophorum* OR 1.6 (1.4-1.9 95CI) and GAS OR 1.7 (1.4-2.2) were positively associated with complications. GGS OR 0.5 (0.3-0.7) and negative culture and PCR OR 0.6 (0.5-0.7) were negatively associated with complications. Associations persisted in adjusted analyses.

Conclusion: In pharyngotonsillitis patients warranting extended work up for *F. necrophorum* and beta-hemolytic *Streptococci*, findings of GAS or *F. necrophorum* were associated with increased complication rates within 30 days.

FUSOBACTERIUM NECROPHORUM-PCR IN PHARYNGOTONSILLITIS – COULD THE CT-VALUE IDENTIFY PATIENTS AT RISK FOR COMPLICATIONS?

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Background: Previously, we investigated tonsillar carriage of *Fusobacterium necrophorum* by PCR and found a high tonsillar carriage rate (21%) in asymptomatic 15-25 year olds, but the same age group is most commonly affected by severe *F. necrophorum* infections. Interestingly, we found Cycle threshold (Ct)-values in asymptomatic carriers to be high (median 29). Possibly, the Ct-value could differentiate between infection and tonsillar carriage, with bacterial load hypothetically being higher on infected tonsils. The purpose of this study was to investigate differences in *F. necrophorum* Ct-values in patients diagnosed with pharyngotonsillitis who did or did not develop complications.

Methods: Patients with pharyngotonsillitis and positive *F. necrophorum*-PCR were enrolled from July 2016 - December 2020 in the Skåne Region, Sweden. Patients with prior complications or antibiotics (30 days) were excluded. Data was retrieved from registries and electronic charts. Patients were grouped by presence of any complication within 30 days, defined as a composite score of peritonsillar or pharyngeal abscess, otitis, sinusitis, sepsis or septic complications, chronic or recurrent tonsillitis (after 15-30 days) or hospitalization. Ct-values were presented with median and interquartile range (IQR) and compared with the Mann-Whitney U-test.

Results: In total, 969 patients had pharyngotonsillitis and positive *F. necrophorum*-PCR. 29% developed complications. There was no difference in Ct-values between patients who did (median 21, IQR 19-25) or did not (median 21, IQR19-26) develop complications (p=0.51).

Conclusion: In pharyngotonsillitis patients warranting extended work up for *F. necrophorum*, no difference in Ct-values between patients who did or did not develop complications was found. Most patients with pharyngotonsillitis had lower Ct-values than previously described in asymptomatic individuals, however factors such as degree of inflammation of tonsils, sampling technique and symptom duration were not accounted for.

GEOGRAPHICAL DIFFERENCES IN TONSILLAR CARRIAGE RATES OF *FUSOBACTERIUM NECROPHORUM* – A CROSS-SECTIONAL STUDY IN SWEDEN AND ZAMBIA

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Background: While *Fusobacterium necrophorum* historically has been considered normal tonsillar flora, recent studies have suggested that carriage occur transiently in adolescence and young adulthood. However, no studies originating from Africa exist. In this cross-sectional study of tonsillar carriage of *F. necrophorum*, the purpose was to evaluate geographical differences in tonsillar carriage rates of *F. necrophorum* in healthy participants aged 15-25 years in Sweden and Zambia and further investigate the age distribution of tonsillar carriage in Zambia.

Methods: Healthy participants were enrolled at study sites in Lund, Sweden and Macha, Zambia. Specimens were obtained by tonsillar swabs and analyzed with real-time PCR for *F. necrophorum* in October-February 2019-20 in Sweden and in March 2020 in Zambia. In Sweden, eligibility criteria were 15-25 years of age, and in Zambia age above 15 years. Exclusion criteria were any sign of throat infection, any antibiotic treatment within the last 4 weeks, or a previous history of tonsillectomy.

Results: In participants aged 15-25 years, tonsillar carriage was more common in Sweden 21/100 (21%) than in Zambia 6/192 (3%), $p<0.001$. In Zambian participants aged above 25 years, tonsillar carriage was rare 1/76 (1%). No difference of carriage rate between genders were identified in either country. In both countries, the tonsillar carriage rate was higher in 20-25 year olds than in 15-19-year olds, yet differences were not statistically significant.

Conclusion: In conclusion, the high rate of tonsillar carriage in participants aged 15-25 years in Sweden has implications on the interpretation of tonsillar findings in patients with pharyngotonsillitis. Interestingly, a geographical difference was found with tonsillar carriage rarely identified in Zambia.

SHORT BLOOD CULTURE TIME-TO-POSITIVITY IN *FUSOBACTERIUM NECROPHORUM* BACTEREMIA IS ASSOCIATED WITH LEMIERRE'S SYNDROME

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Background: Short blood culture time-to-positivity (TTP) has been shown to correlate with infective endocarditis, yet TTP has never been investigated in *Fusobacterium necrophorum* bacteremia. Since Lemierre's syndrome occurs after an oropharyngeal infection with the development and subsequent embolization of septic thrombophlebitis of the internal jugular vein, it could also be considered an endovascular infection, similar to infective endocarditis. The purpose of this study was to investigate if Lemierre's syndrome was associated with shorter TTP in *F. necrophorum*-bacteremia.

Methods: Patients with *F. necrophorum* bacteremia in the Skåne Region, Sweden from January, 2012 to December, 2020 were enrolled. Exclusion criteria were lack of data on TTP, non-immediate incubation of blood cultures, and polymicrobial growth in the anaerobic bottles. TTP was defined as the time from start of incubation to the first signal of positivity. From 2012-2014 the BacT/ALERT® blood culture system (bioMérieux, Inc.) was used in the Skåne Region with blood culture cabinets available only at the tertiary hospital of the region. From 2015, the BACTEC™ FX blood culture system (BectonDickinson) were introduced at all five major hospitals in the region, along with blood culture cabinets for immediate incubation. Clinical data was collected through electronic chart review. Lemierre's syndrome was defined as a positive blood culture with *F. necrophorum*, a preceding oropharyngeal infection, and a radiologically confirmed diagnosis of neck venous thrombosis or signs of septic pulmonary embolism.

Results: 41 episodes of *F. necrophorum* bacteremia were analyzed, of which 15 (37%) occurred in patients with Lemierre's syndrome. TTP was significantly shorter in patients with vs. without Lemierre's syndrome 21 h (17–25 95CI) vs. 27 h (23–31 95CI) (p=0.03, Student's t-test).

Conclusion: In patients with *F. necrophorum* bacteremia, patients with Lemierre's syndrome had shorter TTP, yet substantial overlap was seen. These findings are in line with the interpretation of Lemierre's syndrome as an endovascular infection.

IDENTIFICATION OF THE IMPORTANT FACTORS OF *FUSOBACTERIUM NUCLEATUM* IN STIMULATING ORAL SQUAMOUS CELL CARCINOMA PROGRESSION *VIA* TN5 TRANSPOSON MUTAGENESIS

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The presence of *Fusobacterium nucleatum* in colorectal cancer (CRC) and oral squamous cell carcinoma (OSCC) has been proved and indicated poor prognosis, and its abundance has shown to increase gradually from stage I to IV. However, knowledge about the mechanism of *F. nucleatum*-induced oral cancer is still limited. In this study, we establish a robust genetic tool to overcome the limitations, in order to identify the critical virulence factors of *F. nucleatum* which play pivotal roles in direct and indirect interactions with OSCC to promote the aggressiveness and invasiveness of the disease.

F. nucleatum transposon library will be created using EZ-Tn5 transposon mutagenesis, pMOD-catP which consists of a chloramphenicol/thiamphenicol resistant cassette will be used as a selection marker for the insertion. Mutant strains will then be used to challenge OSCC cell line, SCC-15, and effects will be observed through the degrees of cell migration, proliferation, invasion, and tumorphere formation. In addition, clinical *F. nucleatum* strains which were previously isolated from OSCC and non-OSCC patients' saliva in Taiwan will also be included in the study.

Our preliminary data has shown the reproducibility of our experimental techniques and protocols as the critical observations published previously. There were 101 and 158 saliva samples collected from OSCC and non-OSCC patients, respectively. However, only 10% of *F. nucleatum* were revivable from the samples. Cell suspension of the clinical isolates was prepared for screening and our preliminary data has shown some of the OSCC clinical strains increased the invasiveness of the OSCC cell line than the non-OSCC group as compared to untreated group. Detailed investigation between the two origins of clinical *F. nucleatum* will be further analyzed, for examples the abundance or variety of cell surface adhesins, and secretion proteins.

By expanding the research on the mechanism behind *F. nucleatum*-dependent OSCC progression, we hope to stimulate the development of novel OSCC prevention, early detection and treatment strategies.

ASSOCIATION OF *FUSOBACTERIUM* SPECIES WITH COLON CANCER AND TRANSLATION TO MOUSE MODELS

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We previously reported that a Malaysian CRC cohort consisting of 45 banked tumor biopsies was enriched for *Fusobacterium nucleatum* by 16S rRNA sequencing compared to paired normal tissue and/or healthy controls, as well as enrichment of *F. necrophorum* and *F. periodonticum* in a meta-analysis of multiple studies. We have now expanded our analysis of this cohort by isolating fusobacterial strains from tumor biopsies and investigating this diverse strain library in mouse models. Because we frequently isolated *Fusobacterium varium* from tumor tissues, we revisited our 16S rRNA amplicon sequencing and found a significant enrichment of *F. varium* in tumors compared to paired normal tissues (p=0.0295). *F. varium* has previously been associated with inflammatory bowel disease, but its association with CRC has thus far been limited to specific geographic locations (e.g. Southern Chinese).

Study of *F. nucleatum* as a potential tumor-inducer has been limited by the high barrier to stable, persistent colonization observed in mice. Indeed, we were unable to colonize antibiotic-treated C57BL/6 wildtype (WT) or $Apc^{MinD716/+}$ (Min) mice with *F. nucleatum,* despite numerous approaches including repeated gavage, use of anesthetized mice, pre-treatment with sodium bicarbonate, or inhibition of gut motility with loperamide. However, we found that WT mice could be colonized by orogastric gavage with sodium bicarbonate followed by *F. varium* strain S046TL-3, and remain colonized for 10 weeks. In Min mice, we observed modest colon tumorigenesis 10 weeks after gavage with *F. varium*, despite gradual clearance of colonization (107 ®102 copies/100ng fecal DNA). CRC-associated *F. varium* strains are known to harbor homologues of *F. nucleatum* virulence factors FadA and Fap2, suggesting a potential common biology between these organisms in the tumor microenvironment. Further studies may identify a critical role for *F. varium* in CRC, as well as identify important similarities and differences between *F. nucleatum* and non-nucleatum *Fusobacterium* spp in the context of CRC.

STUDY OF *FUSOBACTERIUM NUCLEATUM* TYPE II FATTY ACID SYNTHASE SYSTEM USING MOLECULAR AND CHEMICAL GENETICS

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Fusobacterium nucleatum is the most commonly isolated microorganism from colorectal cancer (CRC) tissue and is associated with increased CRC progression. Specifically*, F. nucleatum* has been shown to contribute to tumor cell proliferation, immune suppression, and chemoresistance. While broad spectrum antibiotics can target *F. nucleatum,* they also contribute to gut dysbiosis, which can have severe adverse health effects. Thus, there is a strong need for the development of Fusobacteria specific antimicrobials. Our goal is to identify anti-*Fusobacterial* molecules that will specifically disrupt *F. nucleatum* association with CRC by inhibiting *F. nucleatum* growth. A previous study in our lab identified a compound that specifically targets the enoyl-ACP reductase (ENR) FabK, which is essential to the Type II Fatty Acid Synthase System that is used by bacteria. Genome analysis indicates that *F. nucleatum* possesses the FabK enzyme and none of the other ENR isoenzymes. Therefore, we suspect inhibition of FabK will lead to specific inhibition of *F. nucleatum*, while having a minimal effect on the normal gut microbiota, which typically possess the ENR isoenzyme, FabI. We have determined the MICs of structural analogs of the FabK inhibitor against *F. nucleatum,* and a small panel of species. Our results indicate that the analogs inhibit *F. nucleatum* growth through the FabK enzyme and has no effect on *Enterococcus faecalis* strains that are expressing FabI, an isoenzyme of FabK. Additionally, these compounds are able to inhibit *F. nucleatum* growth in the presence of exogenous fatty acids, indicating that *F. nucleatum* will be unable to bypass inhibition of FabK by incorporating exogenous fatty acids. We plan to use these FabK inhibitors as chemical probes to explore *F. nucleatum* lipid biology.


Anaerobe 2022

Friday, July 29 **Microbiome**

1230-1330 Poster Session I: Microbiome

Posters will be presented in Poster Session I
Friday, July 29 1230-1330.

Friday, July 29 — Gut Microbiome

1230-1330 Poster Session I: Gut Microbiome

Posters will be presented in Poster Session I
Friday, July 29 1230-1330.

COMPARATIVE METAGENOMIC ANALYSIS ON FECAL MICROBIOME OF PREGNANT GOAT

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Food animals, including small ruminants, are a primary source of foodborne infections. Further, manure from food animals can, and do make their way into the local environment, where it can lead to contamination of food and water. Consumers are increasingly supporting small ruminant production systems for financial reasons and as a food source, yet limited data are available about the safety of food from these animals. The aim of this study was to examine the impact of pregnancy in goats on the ecology of their gastrointestinal microbiomes and determine the presence and distribution of potential the foodborne bacteria related to pregnancy.

For that, shotgun and 16S metagenomic sequencing using Illumina MiSeq was performed on the feces collected from 5 does, three that were pregnant and two that were not. The fecal samples were collected once a week over 6 weeks with total 28 samples. The microbiome populations in the pregnant were compared during late pregnancy and after delivery. Sequencing data was analyzed using the MG-RAST analyses pipeline and QIIME-2. Overall, the phyla Bacteroidetes and Firmicutes corresponded to 42% and 39% of the taxa detected, respectively. However, after delivery, the relative abundance of Firmicutes increased to approximately 50%. There were approximately ~ 300 variable species identified among all the analyzed samples. Notably, does following delivery had lower abundances of *Campylobacterales* and *Enterococcaceae*. These data suggest that the pregnancy in small ruminants may play an important factor shaping overall gut microbiomes in goats. This study provides initial data to help better understand the influence of pregnancy in small ruminants and the changes in microbiome structure associated with pregnancy and delivery, which has potential effects to cause foodborne illness.

INSIGHTS INTO THE GENOME OF FIRST CLINICAL MULTIDRUG-RESISTANT ISOLATE OF *BACTEROIDES NORDII*

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A statement of the purpose: *Bacteroides nordii*, is a member of pathogenic *B. fragilis* group. Thus, it is of great interest to study the genome evolution of this species. To the best of our knowledge, no detailed genome study is available which characterized *B. nordii* at the genetic level and explored its role as a potential pathogen.

Method: We isolated a multidrug-resistant (MDR) strain (PGMM098) of *B. nordii* (resistant to clindamycin, metronidazole, piperacillin-tazobactam and cefoxitin) from the pus sample and subjected it to the whole genome sequencing using Illumina technology. Raw reads were preprocessed using PRINSEQ and de novo assembly was conducted using CLC Genomics Workbench. RAST server was used for annotation of the draft genome. To predict the antimicrobial resistance (AMR) genes, CARD and ResFinder databases were used. HGTector and KaKs_Calculator were used to investigate the horizontal transmission and selective pressure (ω) operating on the AMR genes.

Results: The draft genome of *B. nordii* PGMM098 has a size of almost 5.7 Mbp. *B. nordii* PGMM098 was found to harbor 6 AMR genes, including *aadS* (streptomycin-resistance), *cfxA3* (class A ß-lactamase), *nimE* (nitroimidazole resistance), *ermF* (macrolide-lincosamide-streptogramin B resistance), *adeF* (efflux transporter periplasmic adaptor subunit), and *tetQ* (tetracycline-resistance). Our further analysis of publicly available genomes predicted 6 more AMR genes in *B. nordii* species. Interestingly, *nimE* gene was found to be present only in our newly sequenced strain. In *B. nordii* PGMM098, *nimE* and *cfxA3* genes were validated using targeted PCR. Towards the AMR evolution in *B. nordii*, the *nimE* gene was predicted to be acquired horizontally and evolved *via* diversifying selection (positive selective pressure, $\omega > 1$). Similarly, *erm*, *tetQ*, and *cfxA3* genes were also found to be under positive selective pressure, however, HGT was not found as the mode of transmission of these genes.

Conclusion: Our study characterized the genome of the first MDR *B. nordii* strain and explored AMR evolution in *B. nordii* using a comparative genomics approach.

ENTEROTOXIGENIC BACTEROIDES FRAGILIS AND *FUSOBACTERIUM NUCLEATUM* IN COLON TISSUES OF PATIENTS WITH COLORECTAL CANCER: A QUANTITATIVE COMPARISON WITH HEALTHY INDIVIDUALS

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It has been suggested that some bacteria may be associated with colorectal cancer (CRC). We aimed to investigate the role of *Fusobacterium nucleatum* and *Enterotoxigenic Bacteroides fragilis* (ETBF) in the etiology of CRC, evaluating their quantities in colon tissues. Colon biopsies of 35 patients and 35 healthy individuals were studied (year 2019) in three age groups (Age 40-49, age 50-74, age ≥75), matching them in term of gender and people number. The quantities (log10 bakteri/25mg) of bacteria were determined by qPCR and specific primer sets: ETBFFw(5′-GAC GGT GTA TGT GAT TTG TCT GAG AGA-3′), ETBFRv(5′-ATC CCT AAG ATT TTA TTA TCC CAA GTA-3′), FnFw(5′-CGG GTG AGT AAC GCG TAA AG-3’), FnRv1(5′-GCC GTG TCT CAG TCC CCT-3’),FnRv2(5′-GCA TTC GTT TCC AAA TGT TGT CC- 3’). When evaluated in terms of gender, *F. nucleatum* quantities were found significantly high in women with CRC than men (p=0.003), and in 50-74 age group *F. nucleatum* quantities were significantly high in women with CRC than controls (p=0.009), but not different between men with CRC and controls (p=0.083). ETBF quantities in women and men with CRC of 50-74 age group were significantly higher than controls (p=0.005, p=0.047). Regardless age and gender, in patients with CRC the quantities of *F. nucleatum* were not different than controls, whereas ETBF quantities were significantly high than controls (p=0.002, p=0.004). CONCLUSION: Between 54-74 ages, ETBF could play a role in the etiology of CRC in both men and women and *F.nucleatum* only in women.

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SPECIES-TARGETED SORTING AND CULTIVATION OF COMMENSAL BACTERIA FROM THE GUT MICROBIOME

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Sequencing studies have highlighted the association of deficiencies in a variety of gut commensal species with various pathological conditions. It is therefore of great interest to use well-characterized strains to complement dysbiotic microbiota. However, this approach is still hampered by the fact that there are usually only few or even no strains available for many species of interest, due to specific nutritive requirements, extreme oxygen sensitivity (EOS), or under-representation in the gut ecosystem. In an attempt to circumvent these limitations, we developed flow cytometry and cell sorting under anaerobic conditions using a modified BD Influx® cell sorter. Viability- and Gram-staining, as well as specific polyclonal antibodies, were investigated as characterization tools. We demonstrated that viability of the EOS species *Faecalibacterium prausnitzii* was preserved during the anaerobic sorting process, while complete loss of viability was observed in normal sorting conditions. Staining procedures had only marginal effects on cultivability. Using antibodies directed against strains that belong to two different phylogroups, we established a collection of 15 strains of the EOS species *F. prausnitzii.* We then focused on the species *Christensenella minuta* that is usually found in very low amount in the human fecal microbiota and were able to establish a collection of 7 strains that belong to this health-related species. These developed tools allow rapid fingerprint of microbiota composition and accurate isolation of EOS bacteria from complex microbial communities. New developments are underway to apply reverse genomics strategies to identify sequences encoding immunogenic proteins in order to produce antibodies, even in the absence of already cultivated strains.

DETECTION OF ANAEROBIC BACTERIAL GROWTH AND FERMENTATION BY BROMOCRESOL PURPLE AND PRESTOBLUE

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The human gut microbiome is a diverse ecosystem consisting of hundreds of anaerobic bacterial species, many of which remain poorly characterized. High-throughput *in vitro* growth characterization of many anaerobic bacterial species has been hindered by a lack of aerobic characterization protocols adapted to anaerobes. Here, we developed two assays to quickly screen novel bacterial isolates for growth and acid production under variable anaerobic conditions. To assay anaerobic bacterial capability to use carbohydrates for growth, we used PrestoBlue (PB) as an indicator for cell viability following a 24-hour incubation period, which reduces inside living cells resulting in a color change. To assay the ability to produce acid from different substrates (i.e. fermentation), bromocresol purple (BCP) was used as a colorimetric indicator of pH change. To validate assay effectiveness, we focused on strain variation of the *Clostridium innocuum* species, a gram-positive, anaerobic, spore former. Six representatives from a larger collection of *C. innocuum* isolates from human fecal material were selected for *in vitro* studies. Isolates were inoculated into basal media supplemented with casamino acids and a single carbohydrate source, such as glucose, or other source. After incubation with PB or BCP, appropriate absorbance readings were recorded for each assay. To validate how well the PB assay represented growth, growth curves based on optical density were recorded during the incubation period. Growth assessed by PB demonstrated that an absorbance ($OD_{568\ nm}$) of 0.5 and above provided a qualitative measure of growth under each substrate. Fermentation capability assessed by BCP provided pH quantitation using absorbance within the range of 4.2-8.4. Importantly, both assays demonstrated differences in substrate use for growth and/or fermentation across *C. innocuum* strains, which corroborated predicted nutrient use from genomic comparisons across a larger set of *C. innocuum* strains. These results support use of these assays as a quick screen to further characterize growth conditions of other bacterial isolates under anaerobic conditions.

LOCALIZED MICROBIALLY-INDUCED INFLAMMATION INFLUENCES CHANGES IN DISTANT HEALTHY TISSUES IN THE HUMAN ORAL CAVITY

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Oral infections are often correlated with systemic health issues in humans. Human hosts fall into three clinical Inflammatory Responder Types (IRTs) associated with microbially-induced oral infections: High-IRT, Low-IRT, and Slow-IRT. Through a highly temporal and multi-omic analysis of microbially-induced inflammation using the experimental gingivitis (EG) model, we observe localized inflammation induced by normal plaque accumulation and maturation results in host mediator and subgingival microbiome changes in distant healthy tooth sites located contralaterally in the mouth. Including significant alterations in pro-inflammatory host mediators IL-8, IL-6, IL1-b, and TNF-a as well as a dysbiotic ecological shift within the subgingival microbiome, represented by an inverse Firmicutes/Bacteroidetes ratio (iFBR). This study provides insight into how distant healthy tissues are affected by localized microbially-induced inflammation in an IRT dependent manner within the human oral cavity.

FAECALIBACTERIUM PRAUSNITZII AT THE CENTER OF THE ONSET OF THE ATOPIC DERMATITIS

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The gut microbiota plays a pivotal role in human physiology and their malfunction has been linked to chronic diseases, including atopic dermatitis and inflammatory bowel diseases. We have been investigating the interrelationship between the aberrant gut microbiota and atopic dermatitis. We recently discovered that subspecies-level misbalance in one of the main gut bacteria, *Faecalibacterium prausnitzii*, underlies the onset and/or progression of atopic dermatitis. *F. prausnitzii* has long been known as one of the most beneficial species in the human gut. However, we isolated subspecies of *F. prausnitzii* that act as pathobionts, enrichment of which in the gut is associated with the disease. In a murine model, the atopic-disease potential caused by the *F. prausnitzii* pathobionts in dams were inter-generationally transferred to their pups, demonstrating the phenomenon similar to the development of atopic dermatitis in human infants. Increased incidences of atopic dermatitis over the past decades are largely due to lifestyle changes and misbalance in the *F. prausnitzii* population is thought to be associated with these changes. While current understanding of *F. prausnitzii*-driven gut microbiology is in its early stage, it will be of tremendous significance in the future to understand the onset and the progression of atopic dermatitis and various chronic diseases.

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EFFECTS OF THE PROBIOTIC *LACTOBACILLUS REUTERI* IN A PRAIRIE VOLE MODEL

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Statement of Purpose: This study aims to investigate the effects of probiotic intake on behavior, neurochemical marker expression and gut composition in a prairie vole model taking advantage of their unusual social behaviors, such as pair bonding.

Methods: Male and female voles were treated with heat-killed or live probiotic *Lactobacillus reuteri* through water intake over a period of 4 weeks. Post-treatment behavior and neurochemical differences were determined and the 16S rDNA V3-V4 region was sequenced from pre- and post-treatment stool samples from each animal. Whole genome shotgun (WGS) sequence was also determined for a subset of voles for better characterization of the gut composition and to supplement the 16s amplicon data. Microbiome data was analyzed using a variation of the differential abundance analysis combining whole group and individual differences along with more standard differential abundance, diversity, and correlation analysis.

Results: Females treated with live probiotic displayed lower levels of social affiliation compared to their heat-killed treated counterparts. No difference was seen in males, however, males generally displayed higher levels of anxiety-like behaviors over females. Live treated females had key differences in neurochemical expression, such as lower vasopressin 1a-receptor but increased corticotrophin-releasing factor expression in the paraventricular nucleus of the hypothalamus. In addition, female live treated voles displayed increased abundance of several noteworthy gut taxa, including *Clostridiales* VE202-01. Interestingly, heat-killed treated females displayed an increased abundance of potentially beneficial taxa such as butyrate-producing Blautia. 16S and WGS sequencing data indicated baseline sex differences in microbiome composition and diversity.

Conclusions: Our data indicate that *L. reuteri* can impact gut composition and the gut-brain axis in a sex-specific manner in the socially monogamous prairie vole. Our data also demonstrates the utility of the prairie vole model for further examining causal impacts of gut microbiota on the brain and behaviors.

METAGENOMICS INSIGHT INTO THE GUT MICROBIOME FUNCTIONAL GENES OF HEALTHY INHABITANTS IN LAGOS, NIGERIA

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The gut microbiota of humans has been implicated in health and disease, but, their roles in healthy adults in Africa is quite unclear. We set to reveal functional roles of the gut microbiome of a rural (n=10) and urban (n=10) population of Lagos State. Collection of sociodemographic and clinical data, fecal DNA extraction, amplicon sequencing, and trimming was done by standard procedures. Functional and antibiotics resistance genes (ARGs) were annotated via Clusters of Orthologous Groups (COG), Eukaryotic Orthologous Groups of proteins (KOG), and Comprehensive Antibiotic Resistance Database (CARD) tools. Potential link between phenotypes was inferred by principal component analysis (PCA). Predominant genera in urban were *Bifidobacterium, Prevotella*, and *Faecalibacterium,* while *Prevotella* and *Faecalibacterium* were in the rural population. We found 12 (urban) and 11 (rural) COG classes of proteins from the gut microbes. Class E for amino acid transport and metabolism was unique to urban mainly 5-enolpyruvylshikimate-3-phosphate synthase (45a%) and Asparagine synthase (30%) denoting a high protein-based diet. Class S was found in both, but more in the rural. Rural also had genes for exo-beta-1,3-glucanase (31%), pyruvate decarboxylase, and thiamine pyrophosphate-requiring enzymes (22%) indicative of carbohydrate-rich diets. KOG revealed 16 of the same class T, denoting signal transduction mechanisms. Uncharacterized protein linked to hypothetical functions was found in both, and 60% of rural gut microbiomes had genes with unknown functions. The COG class clustered with marital status, weight, and sex, then KOG with age. CARD showed ARGs (urban 90%; rural 10%) and all were *Escherichia coli* 16S rRNA (rrsB) mutation conferring resistance to streptomycin with gene variant model A523C n/a to aminoglycoside antibiotics. Differences seen between sites and predicted functional roles appear to be driven by sociodemographic and clinical properties. The ARGs observed in the urban population has public health concern and raises issues over the emergence of antibiotic resistance.

Keywords: Functional genes, metagenomics, Nigeria, Gut microbiome, West Africa

EFFECTS OF NANO ZINC OXIDE AND *STREPTOCOCCUS ORALIS* ON THE GROWTH AND BIOFILM FORMATION OF *STREPTOCOCCUS MUTANS*

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Purpose: The aim of this study was to evaluate the effects of nanoparticle zinc oxide (nZnO), currently used in dentistry, and *S. oralis* on the growth and biofilm formation by *S. mutans*.

Methods and Results: Mono and dual culture of *S. mutans* and *S. oralis* (10^5-10^6 CFU/mL, respectively) were prepared in artificial saliva medium (BMM) containing 1% sucrose and subinhibitory concentration (3.2 mg/ml) of nZnO using 48-well microtiter plates. Before use, sonication was employed for 15 min at 48 W and 35 kHz to disperse the nZnO. All cultures were grown anaerobically with 110 rpm agitation at 37°C for 24 h. The 24 h growth dynamics were determined spectrophotometrically at an optical density reading of 600 nm by mineral oil overlay method. The phenol-sulfuric acid method was used to quantitate extracellular polysaccharide (EPS) in biofilm. A colorimetric assay using 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) was employed to measure the viability of bacteria in the biofilm along with a bacterial plate counting method. *S. mutans* were selectively counted from the dual culture, with *S. oralis* using agar plates containing 4 U/ml of bacitracin. Biofilm formation, EPS production, MTT assay, and CFU of *S. mutans* were decreased by *S. oralis* in dual culture but not the opposite way. The addition of nZnO increasingly inhibited the biofilm formation, EPS production, MTT assay, and CFU of *S. mutans* in the dual culture, but the modes of inhibition were different with nZnO and *S. oralis*. *S. oralis* inhibited EPS production, which resulted in biofilm reduction, while nZnO inhibited the growth of *S. mutans,* but not EPS production.

Conclusion: Mixed cultures of *S. mutans* and *S. oralis* and those containing subinhibitory concentrations of nZnO inhibited biofilm formation, EPS production, MTT assay, and CFU of *S. mutans*.

COMPARING MICROBIAL COMPOSITION IN LUNG CANCER TISSUE USING 16S RIBOSOMAL RNA AMPLICON SEQUENCING AND ANALYSIS OF THE CANCER GENOME ATLAS SEQUENCING DATA

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The microbiome appears to be a key driver of disease, including cancer. However, it is unclear whether bioinformatic analyses using differing methods yield similar results. Two large, high-profile studies of cancer microbiomes, Nejman *et al, Science* 2020;368:973 and Poore *et al, Nature* 2020;579:567, analyzed, respectively, 16S rRNA amplicon sequencing of frozen and fixed tissues *vs* bioinformatic extraction of microbial sequences from The Cancer Genome Atlas Sequencing (TCGA) RNA (derived from polyA+ RNA sequences) and DNAseq data. Bioinformatics methods also differed. Herein, we examined the results of each study using the data reported for lung cancer samples (N=243 and 1263 lung cancers, respectively) to determine the extent to which the two studies reported consistent taxonomic results for the bacterial microbiota of lung cancer. Taxonomic concordance between the two studies was 39% (Phylum), 36% (Class), 36% (Order), 40% (Family) and 19% (Genera). Species level data was only able to be reported using 16S rRNA amplicon sequencing. Despite extensive efforts within both studies to computationally limit contaminants, our analysis indicated the final reported datasets appear to remain highly contaminated with environmental bacteria. When only concordant taxa data were analyzed across the two studies, each taxa level displayed significant between study correlation [P value range: <0.001 (Class, Order, Genus) to <0.03 (Phylum, Family)] with Spearman rank-order correlation coefficients ranging from 0.19 (Family) to 0.65 (Class). In summary, reported lung cancer microbiomes differed markedly between two analytical approaches. While the patient cohorts differed between the studies potentially accounting for some of the data variation, these results provide further evidence that results across different computational studies must be carefully reviewed for consistency, completeness of contaminant removal and biologic relevance. Validation of the lung cancer microbiome in additional cohorts is needed.

ARABINOXYLAN BRANCHING STRUCTURE GOVERNS COMMUNITY COMPOSITION AND METABOLISM OF FERMENTING HUMAN GUT MICROBIOTA

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Arabinoxylans (AXs) are complex, hemicellulosic polysaccharides, and common dietary fibers that are fermented by colonic bacteria. AXs have complex molecular structures, including large molecular sizes, multiple diverse sugar composition and glycosidic linkage patterns, and diverse branching chains that decorate the b-1,4-linked xylan backbone. These structural characteristics can be the basis for selective effects for specific taxa within the microbiome, and theoretically, the stringency of these responses may influence the ability of an AX to specifically target certain gut microbes. Herein, we hypothesized that simplification of a branching fiber structure would reduce the microbial diversity sustained by the polysaccharide due to the removal of the corresponding specialist niches, which are afforded by more complex structure. To test this hypothesis, we applied a two-step enzymatic modification to remove arabinose branches from the original sorghum arabinoxylan (SAX), without much reduction in molecular size. Both native and modified SAXs were subjected to a 7-day *in vitro* sequential passage fecal fermentations with inocula from three separate individuals, and for which we measured community composition (by 16S amplicon sequencing) and intermediate metabolic outcomes (gas, pH, and SCFAs) daily. The detailed chemical structures and molecular size distribution of the two SAX substrates were determined by GC with partial methylation alditol acetate derivatization and high-pressure size exclusion chromatography (HPSEC). Our results suggest that the phylotypes selected from the two SAX variants were shared at the operational taxonomic unit (OTU) level among all three individual's microbiota, implying that substrate molecular structure deterministically governs microbial succession. Furthermore, we found that SCFA production was precisely predicted by branching linkages, suggesting a hypothetical approach to manipulate *in situ* gut metabolites predictably via highly specific, and potentially modified, fiber structures.

Saturday, July 30 — Young Investigator's Presentations

1230-1330 Young Invetigator's Presentations

Posters will be presented in Young Investigator's Presentations Saturday, July 30 1230-1330.

FUSOBACTERIUM NECROPHORUM OUTER MEMBRANE PROTEINS AS VACCINE CANDIDATES AGAINST FUSOBACTERIAL INFECTIONS

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Fusobacterium necrophorum is a Gram-negative, anaerobic opportunistic pathogen, which causes necrotic infections in cattle. Its necrotic infections resulting in liver abscess, foot rot, and calf diphtheria imparts a high financial impact on the U.S. feedlot industry. Antibiotic administration is the mainstay to treat these infections, but antibiotic-resistance is unavoidable. Thus, a vaccine could be the best alternative prophylaxis. In most Gram-negative pathogenesis, bacterial attachment to the host cell is a crucial step, therefore Outer Membrane Proteins (OMPs) is an active study area for vaccine development. The pathogenic and immunogenic role of the *F. necrophorum* OMPs have not been studied thoroughly. Here, we have identified high binding affinity adhesins (17-OmpH and 22kDa-OmpA OMPs), and 67kDa cell surface proteins (CSP) using binding assays and pull-down assays, respectively, with bovine adrenal gland endothelial (EJG) cells. The corresponding genes were sequenced and cloned in the expression plasmids. The recombinant proteins were purified, and polyclonal antibodies were generated against these OMPs. The efficacy of these polyclonal antibodies was studied through *in vitro* adhesion inhibition assay. Our results showed combinatorial treatment using 17kDa -OmpH and 22kDa- OmpA antibodies significantly decreased bacterial adhesion to the host cells. Also, individual polyclonal antibody treatment raised against 67kDa -CSP exerted a significant bacterial adhesion inhibition. Overall, the combinatorial inhibitory effect on adhesion was more prominent. We identified OMPs with potential roles in bacterial attachment to the host cell, which could serve as candidates for vaccine development. Further *in vivo* investigation is required to confirm the role of OMPs and develop the OMP-based vaccine against fusobacterial infection.

EXAMINING *ACINETOBACTER CALCOACETICUS'* ABILITY TO GROW IN INTESTINAL CONDITIONS AND MODULATE THE GUT EPITHELIUM

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Background: *Acinetobacter* is characterized as a group of environmental bacteria that can occupy several ecological niches, including the mammalian intestine. *Acinetobacter* has been identified in the human fecal microbiota, and it is speculated that the gut can serve as a reservoir for this pathobiont. Given the increasing prevalence of this pathogen in the clinical setting and the lack of information surrounding its colonization properties, we sought to examine the ability of *A. calcoaceticus* strains to survive environmental stressors found in the gut and identify key nutrients used to support its growth. Using 2 commercially available strains and 4 clinical isolates, we found that all *A. calcoaceticus* strains were fairly resistant to high osmolarity (0.1, 0.5 and 1 M NaCl), ethanol (1, 2.5, and 5%) and hydrogen peroxide (0.05, 0.1 and 0.2%). In general, the clinical isolates were more resistant than the commercial strains. All strains were able to grow in pH 7, 6, 5 and 4 media; although a reduction in growth was observed at the lower pHs. Biolog phenotypic microarrays in minimal media lacking glucose revealed that all strains could use the following sugars: glucose, L-arabinose, D-galactose, D-mannose, D-fructose, GluNAC, and trehalose. With found strain-dependent growth with maltose, D-melibiose, sucrose, D-cellobiose, maltotriose, and other sugars. In terms of acids, all strains used a-keto-glutaric acid, and most used pyruvic acid to support its growth. *A. calcoaceticus* strains could also use the amino acids L-glutamine and L-aspartic acid as an alternative to a carbon source. Finally, we sought to examine the interaction between *Acinetobacter* and the gut epithelium. Incubation of live *A. calcoaceticus* strains with inside-out intestinal organoids significantly increased pro-inflammatory cytokines (TNF, KC/IL-8, MCP-1 and IL-1α,) and decreased MUC2 and MUC13 transcripts, without altering tight junctions. Collectively, these data demonstrate that *A. calcoaceticus* is well adapted to the gastrointestinal tract and points to the potential for *Acinetobacter* to influence the gut epithelium.

THE ROLE OF A *CLOSTRIDIOIDES DIFFICILE* P-TYPE ATPASE IN INTRACELLULAR IRON BIOMINERALIZATION AND ITS IMPACT ON CELLULAR PHYSIOLOGY AND PATHOGENESIS

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Clostridioides difficile is the leading cause of nosocomial and antibiotic-associated intestinal infections. Antibiotics, the primary treatment for *C. difficile* infection (CDI), further disrupt the microbiota and lead to infection relapses in 25% of patients. This underscores the urgency to identify alternative strategies for CDI. Our study focuses on the battle for the essential nutrient metal iron between the vertebrate host and *C. difficile*. Iron is indispensable for all forms of life, but toxic at elevated levels. To survive and propagate within the host, bacterial pathogens have evolved comprehensive iron uptake, storage, and detoxification strategies to maintain iron homeostasis. However, these iron homeostatic systems are largely undefined in *C. difficile*. Unlike other well studied Gram-positive bacteria, such as *Bacillus subtilis* and *Staphylococcus aureus*, *C. difficile* is an obligate anaerobe and may possess distinct iron homeostatic strategies to accommodate its strict anaerobic lifestyle. Indeed, we discovered that *C. difficile* undergoes an intracellular iron biomineralization process and produces iron-rich granules (ferrosomes) to maintain iron balance during transient iron overload. Additionally, we found that a P_{1B6}-ATPase transporter (FezB), regulated by both iron and the ferric uptake regulator Fur, is required for ferrosome formation. To further study this biological process, we employed state-of-the-art technologies including scanning transmission electron microscopy paired with energy dispersive x-ray spectroscopy, freeze-substitution, and cryogenic electron microscopy. We uncovered that ferrosomes are iron- and phosphorus-rich granules, localized inside *C. difficile*, and bound by membrane vesicles. Based on these findings, we propose a model whereby (i) FezB transports iron into ferrosomes and interacts with other factors during this process, (ii) ferrosomes serve as a novel iron storage strategy to alleviate iron overload and oxidative stress, (iii) stored iron in ferrosomes is released through a specific mechanism to support growth under iron limitation, and (iv) ferrosomes are produced in the inflamed gut to combat host iron sequestration and required for bacterial survival during CDI. This study redefines the concept of bacterial iron storage and creates a framework for developing effective antimicrobial therapeutics to combat CDI.

DIFFERENTIAL INHIBITION OF C. DIFFICILE BY MICROBIAL DERIVED SECONDARY BILE ACID LITHOCHOLATE AND ITS DERIVATIVES RESULT IN DIVERSE MECHANISM OF ACTIONS

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Disruption of the indigenous gut microbiota and the loss of microbial derived secondary bile acids are associated with increased susceptibility to *C. difficile* infection (CDI). Previous work has shown that secondary bile acid isolithocholate (iLCA), a lithocholate (LCA) derived isomer has potent inhibitory activity against *C. difficile* strain R20291. Our objective was to further characterize LCA and its derivatives to determine if and how they can inhibit toxin production in *C. difficile,* while sparing commensal members of the gut microbiota. We tested the inhibitory activity of three LCA derivatives iLCA, 3-oxo-LCA, and iso-allo-LCA (iaLCA) with *C. difficile* strain R20291 and a commensal gut microbiota panel (*C. scindens, C. hylemonae, C. hiranonis, B. thetaiotaomicron, L. acidophilus, L. gasseri, B. fragelis, B. stercoris, B. thetaiotaomicron, E. coli*, and *B. longum* subsp infantis). To determine the mechanism of action by which LCA and its derivatives inhibit *C. difficile*, we performed a series of experiments to identify the minimum inhibitory concentration (MIC), bacterial killing, membrane integrity disruption, and effects on toxin expression. Further, Caco-2 cell apoptosis, viability, and permeability assays were used to determine the cytotoxicity of these compounds against the host. *C. difficile* growth was found to be strongly inhibited by iLCA, 3-oxo-LCA, and iaLCA, as reflected by low MICs ranging from 0.03 mM to 0.1 mM. In comparison, a majority of commensal gut microbes tested from the panel did not experience inhibition of growth in the presence of high concentrations of the bile acids (1.25 mM). iLCA and iaLCA had bactericidal activity against *C. difficile* and significantly damaged the bacterial membrane integrity of *C. difficile* at 0.5X MIC. Finally, LCA decreased expression of toxins tcdA and tcdB over a 48-hour period. These results suggest that both iLCA and iaLCA have potent inhibitory activity against *C. difficile,* while sparing commensal members of the gut microbiota. Although iLCA and iaLCA are both epimers of LCA, they have distinct mechanisms for inhibiting *C. difficile.* The properties of iLCA and iaLCA suggest their potential use as novel compounds that can target *C. difficile,* while sparing gut commensals that are important for colonization resistance. Future efficacy studies in a mouse model of CDI are warranted.

TRANSCRIPTOMIC ANALYSIS OF *C. DIFFICILE* IN THE PRESENCE OF A *LACTOBACILLUS ACIDOPHILUS* CL1285, *L. CASEI* LBC80R AND *L. RHAMNOSUS* CLR2 SUGGESTS DOWNREGULATION OF CRITICAL GENES INVOLVED IN VIRULENCE

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Clostridioides difficile infections (CDI) result from antibiotic use and cause severe diarrhea which is life threatening and costly. A specific probiotic containing *Lactobacillus acidophilus* CL1285, *L. casei* LBC80R, and *L. rhamnosus* CLR2 (Bio-K+) has demonstrated clinical benefits in preventing CDI and displays a strong inhibitory effect on the growth of several nosocomial *C. difficile* strains. Here, transcriptomic analysis revealed novel mechanisms by which Bio-K+ could impair *C. difficile* virulence.

Methods: The hypervirulent strain *C. difficile* R20291 was co-cultured anaerobically with Bio-K+ probiotic strains in bioreactor in BHI media for 24 h at 37°C. Parameters such as agitation, pH, and anaerobiosis were controlled and total gene expression was evaluated by transcriptomic analysis using Illumina NextSeq500, obtaining around 20 M single-end reads per sample. Statistical comparisons using DESeq2 were performed in order to determine if the groups were significantly different. In a second step, genes overexpressed/repressed with particular interest on virulence genes were validated by a qPCR approach.

Results: A total of 1,156 genes were differentially expressed (DEGs), including 620 underexpressed and 536 overexpressed genes in the co-cultures of *C. difficile* with Bio-K+. The majority of DEGs were concentrated on carbohydrate, amino acid, energy, and transmembrane transport metabolic pathways. However, genes related to motility, adhesion, biofilm production, quorum sensing which are involved in CD virulence, have been underexpressed. All the flagellar genes were underexpressed, as well as some QS genes such as *LuxS,* all of them have a role within biofilms. Several genes related to spore viability and germination were underexpressed.

Conclusions: These results suggest that the combination of *L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 interferes with *C. difficile* pathogenesis through modulation of genes involved in *C. difficile* virulence. These results shed some new lights to better understand the mechanisms of action of Bio-K+ probiotic product to prevent CDI in antibiotic treated patients.

KLEBSIELLA PNEUMONIAE IN THE COLONIC MUCUS LAYER INFLUENCES *CLOSTRIDIOIDES DIFFICILE* PATHOGENESIS

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Despite new technologies, *Clostridioides difficile* infection remains a pressing health care burden. *C. difficile* infection commonly follows antibiotic administration, which disrupts the normal gut microbiota architecture. In the setting of antibiotics, *Klebsiella* spp. can also thrive. *Klebsiella* infection secondary to *C. difficile* infection has been noted in the literature, suggesting that *Klebsiella* may act in concert with *C. difficile* in the antibiotic-treated gut. We hypothesized that *K. pneumoniae* may enhance *C. difficile* traits required for colonization. To test this hypothesis, we utilized 36 *K. pneumoniae* strains, both commercially available and clinical isolates, with four *C. difficile* strains that produce various levels of toxins (R20291, 630, M68, and non-toxic). We observed that co-cultures of *K. pneumoniae* and *C. difficile* had increased growth when compared to either bacteria alone. Moreover, supernatant supplementation confirmed that metabolites could cross-fed the other species. To examine toxin production, we treated LifeAct labeled Vero cells and monitoring cell rounding by live cell imaging. We observed that co-cultures of bacteria had decreased cell rounding, indicating that *K. pneumoniae* prevents *C. difficile* nutrient starvation. Although *K. pneumoniae* is known to produce robust biofilms, biofilm production has reduced in the presence of *C. difficile*, indicating that biofilm production is not a key component of *K. pneumoniae*-*C. difficile* interactions. Finally, to examine the interaction of these microbes with the epithelium, inside-out colonic organoids were treated with live *C. difficile* and/or *K. pneumoniae*. RNA sequencing revealed that co-cultures of bacteria increased pro-inflammatory responses in the organoids compared to either bacteria alone. These results suggest that *K. pneumoniae* acts synergistically with *C. difficile* and promotes colonic inflammation. We suspect that interactions between *K. pneumoniae* and *C. difficile* allow these bacteria to better colonize the intestine early during infection.


Anaerobe 2022

1315-1415 Poster Session II: Clinical

Posters will be presented in Poster Session II
Sunday, July 31 1315-1415.

Sunday, July 31 — Clinical

1315-1415 Poster Session II: Clinical

Posters will be presented in Poster Session II
Sunday, July 31 1315-1415.

ANTIBACTERIAL ACTIVITIES OF CATESTATIN, CECROPIN A, NISIN, AND TEMPORIN A PETIDES ON CLINICALLY IMPORTANT ANAEROBIC BACTERIA

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Today antimicrobial peptides are increasingly coming into the focus as new treatment strategies for bacterial infections. We aimed to determine the antimicrobial activities of catestatin, nisin, cecropin A and temporin A peptides on *Bacteroides fragilis* ATCC 25285, *Prevotella melaninogenica* ATCC 2584, *Cutibacterium acnes* ATCC 6919, *Peptostreptococcus anaerobius* ATCC 27337, and *Peptostreptococcus stomatis* DSM 17678 reference strains. The antibacterial activities of peptides were assigned by agar dilution method to determine their minimal inhibitory concentrations (MIC). Peptides were dissolved and diluted, final concentration of catestatin and cecropin A were 1 µg/mL-500 µg/mL, temporin A was 5 µg/mL-500 µg/mL and nisin was 0,4 µg/mL-40 mg/mL. Tests were performed on Brucella agar supplemented with vitamin K1, hemin, L-cystein and hemolyzed sheep blood and incubation was made under anaerobic conditions provided by GasPack (BD) on anaerobic jars (Oxoid) during 72 hours at 37°C. The evaluation was made upon EUCAST criteria. Only cecropin A was found active to *B.fragilis* (MIC 50µg/mL).

Additionally cecropin A and nisin were found active to *P. anaerobius* (MIC 8µg/mL and 40 mg/mL respectively), *P. melaninogenica* (MIC 16µg/mL,400µg /mL), *C. acnes* (MIC 8µg/mL, 40 mg/mL) and *P. stomatis* (MIC 4µg/mL,40 mg/mL). Temporin A was found active only to *P. anaerobius* (MIC 500µg/mL) and catestatin was not found active to tested reference strains. In conclusion cecropin A and nisin were found to have a good *in vitro* activity to tested reference strains. They may be a new treatment hope against infections caused by anaerobes resistant to antibiotics. It would be useful to conduct further research with these peptides.

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Project ID: 32540

INVESTIGATION OF *CUTIBACTERIUM ACNES* AND *BACTEROIDES FRAGILIS* IN PROSTATE TISSUES OF PATIENTS WITH PROSTATE CANCER: A QUANTITATIVE COMPARISON WITH HEALTHY INDIVIDUALS

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Studies in recent years have shown that *Cutibacterium acnes* may have an effect in prostate carcinogenesis, and the effect of *Bacteroides fragilis* is still being researched. The aim of this study was to investigate the role of *C.acnes* and *B. fragilis* in the etiology of prostate cancer (PC) comparing their quantities in prostate biopsies of patients and healthy controls (HC). Prostate biopsies of 60 patients with PC (Age:64.08±6.66) and 60 individuals without cancer and/or any other pathology (Age: 61,85 ± 5,81) were studied (year 2019-2020) in two age groups (Age 55-65, age 66-75) matching them in term of people number. Bacterial DNA were extracted with QIAamp DNA Mini Kit (Qiagen). The quantities (log10 bakteri/25mg) of *C.acnes* and *B.fragilis* in biopsies were investigated by qPCR on a Rotor-Gene Q 5plex HRM (Qiagen) using microbial DNA qPCR Kits (BBID00285A, BBID00051A, Qiagen) according to manufacturer instruction. *C. acnes* was detected in biopsy samples of 58 (96.6%) patients with PC and in 39 (65%) of HC ($p<0.001$), *B .fragilis* was detected in biopsy samples of 9 (15 %) patients with PC and in 4 (6.7%) of HC ($p=0.142$). Only the quantity of *C. acnes* in PC patients between the ages of 55-65 was significantly high than HC ($p=0.002$) and a significant difference was not found in the quantities of *B.fragilis* between PC patients and HC of both age groups ($p=0.211$). In conclusion, our findings suggest that *C. acnes* may play a role in the etiology of PC, especially in patients between the ages of 55-65, and for *B. fragilis,* we can't speculate any liason.

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THE ROLE OF ANAEROBES IN THE PATHOGENESIS OF CHRONIC RHINOSINUSITIS (CRS)

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Background: Chronic rhinosinusitis (CRS) is characterized by complex bacterial communities that incite persistent inflammation and airway damage. Mucin-degrading anaerobes (MDA) are predominant during the early phase of acute rhinosinusitis in a rabbit model and may provide carbon source nutrients (e.g. short-chain fatty acids, SCFAs) to bacterial pathogens observed after 12 weeks of chronic infection. The objective of this study is to evaluate the capability of MDA to contribute to the growth of *Pseudomonas aeruginosa*.

Methods: Rabbit rhinosinusitis was induced by blocking the sinus cavity for 2 weeks to create an anaerobic environment for MDA. Control and sinusitis mucus were collected and co-cultured with PAO1 strain of *P. aeruginosa* for 72 hours and colony forming units were determined. Targeted quantification of SCFAs in control and sinusitis mucus was performed via high performance liquid chromatography.

Results: Colony counts per tube were significantly higher in those tubes contained with the mucus samples from week 2 (8.4x109+/-4.8x107) compared to tubes containing control mucus (1.4x109+/-2.0x107) or no mucus (1.5x109+/-2.1x107) ($p<0.0001$). Acetate concentrations were significantly greater in the rabbit mucus samples collected on week 2, relative to day 0 (4.13+/-0.53 vs 1.94+/-0.44 mM, $p<0.01$). All SCFAs were significantly higher in CRS compared to controls in human (acetate, $p<0.05$; propionate, $p<0.0001$; butyrate, $p<0.01$).

Conclusion: Given that SCFAs are exclusively derived from bacterial fermentation, our evidence suggests a critical role for mucin-fermenting bacteria in generating carbon-source nutrients for pathogenic bacteria. MDA may contribute to the development of CRS by degrading mucins, thus providing nutrients for potential pathogens like *P. aeruginosa*.

BACTERIAL TAXA CONCENTRATIONS AND GUT GVHD SEVERITY FOLLOWING TRANSPLANT

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Following allogenic hematopoietic cell transplant (allo-HCT), patients are at risk of developing gut graft-versus-host disease (GvHD). Low gut bacterial diversity has been associated with GvHD severity. Anaerobic bacteria in the *Lachnospiraceae* family, specifically *Blautia* species, have been associated with reduced GvHD related mortality. The role of other prominent gut bacteria, such as *Bacteroides* species, in gut GvHD is unclear. Here, we measured concentrations of *Lachnospiraceae, Blautia,* Bacteroidetes, select Bacteroidales, and human-associated *Bacteroides* species in patients undergoing allo-HCT.

Stool samples from 306 allo-HCT patients were collected pre- and post-transplant on days 30 and 60. GvHD gut stage was assessed as 0-1 (none-mild) or 2-4 (moderate-severe). Quantitative PCR assays were designed targeting regions of the 16S rRNA gene. Amplicon specificity was confirmed using post-run melt curve analysis. Concentrations of *Lachnospiraceae* were higher ($p=0.016$) in patients with stage 0-1 ($6.78x10^7$ copies/swab) vs. stage 2-4 gut GvHD ($1.84x10^7$) on day 60. Similarly, a trending positive association for *Lachnospiraceae* ($p=0.088$) was found on day 30. Higher concentrations of *Blautia* species were noted in patients with stage 0-1 ($3.33x10^6$ d30, $1.05x10^7$ d60) vs. stage 2-4 gut GvHD ($1.35x10^5$ d30, $1.41x10^6$ d60) on day 30 ($p=0.026$) and 60 ($p=0.014$) post allo-HCT. Lower concentrations of both *Lachnospiraceae* ($1.89x10^7$) and *Blautia* ($2.30x10^6$) were observed, regardless of GvHD stage, on day 30 vs. pre-transplant ($p<0.001$) or day 60 ($p<0.001$), likely reflecting impact of antibiotics during neutropenia following allo-HCT. Interestingly, levels of Bacteroidetes, Bacteroidales, and *Bacteroides* remained consistently high on day 30. There were no differences in concentrations of Bacteroidetes, Bacteroidales, and *Bacteroides* among patients with stage 0-1 vs. stage 2-4 gut GvHD across all time points.

Higher concentrations of *Lachnospiraceae* and *Blautia* in the gut following transplant were associated with less severe gut GvHD. Further work is needed to determine whether these bacteria are markers or drivers of less severe GvHD.

PREVALENCE AND CONCENTRATIONS OF 4 *GARDNERELLA* SPP. GROUPS IN BACTERIAL VAGINOSIS

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Background: Bacterial vaginosis (BV) is a vaginal condition marked by high bacterial diversity. *Gardnerella vaginalis* has historically been implicated in the development of BV, though it is also detected by PCR in more than 50% of women without BV. The *Gardnerella* genus, once thought to consist of only *G. vaginalis*, has recently been shown to encompass several unique species. We sought to compare the prevalence and concentrations of select *Gardnerella* spp. in women with and without BV to determine if particular species are better markers of BV.

Methods: From October 2012-June 2015, vaginal swabs were collected from 251 women (101 with BV, 150 without BV) attending the Public Health–Seattle & King County Sexual Health Clinic. Women were considered BV positive when at least 3 of 4 Amsel criteria were met. Four quantitative PCR (qPCR) assays were designed targeting the cpn60 genes of *G. vaginalis* and genome sp. 2, *G. piotii* and genome sp. 3, *G. swidsinskii*, and *G. leopoldii*. A qPCR assay targeting the 16S rRNA gene of the *Gardnerella* genus was also used to measure the prevalence of *Gardnerella*.

Results: Concentrations of *G. vaginalis*/genome sp. 2 (4.1E8 *vs* 8.0E7 gene copies/swab), *G. piotii*/genome sp. 3 (1.0E8 vs 5.4E7), *G. swidsinskii* (3.8E9 *vs* 5.6E8), *G. leopoldii* (3.8E9 vs. 5.6E8), and *Gardnerella* (1.1E9 *vs* 1.4E8) were significantly higher in women with BV compared to women without BV (each $p<0.001$). Using the presence of each targeted species, the sensitivity and specificity for each assay in assessing BV was as follows: *G. vaginalis*/genome sp. 2 (98%, 49%), *G. piotii*/genome sp. 3 (91%, 56%), *G. swidsinskii* (82%, 57%), *G. leopoldii* (64%, 75%), and *Gardnerella* (99%, 25%). Women with BV had a higher number of *Gardnerella* species detected compared to women without BV (3.4 *vs* 1.6, $p<0.001$).

Conclusion: Prevalence of any single *Gardnerella* spp. did not accurately predict BV status, but women with a higher number of *Gardnerella* spp. detected were more likely to have BV. This suggests that BV not only reflects a state of high overall bacterial diversity, but one with high *Gardnerella* species diversity.

ANTIMICROBIAL RESISTANCE IN *CLOSTRIDIUM* SPP. ISOLATES FROM SKIN, SOFT TISSUE AND BONE INFECTIONS IN A COSTA RICAN TRAUMA HOSPITAL, 2018 – 2019

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Clostridium sp. is one of the most frequent anaerobes isolated from skin, bone, and soft tissue Clinical samples and these infections are common from hospital trauma services. Knowledge of antimicrobial resistance is recommended for optimal treatment strategies.

Seventy-two isolates of *Clostridium* sp. were obtained of skin, bone, and soft tissue of complicated infections of patients of a trauma hospital from Costa Rica. The isolates were identified by MALDI-TOF and the antimicrobial susceptibility of most frequent isolates (*Clostridium perfringens, Clostridium tertium, C. sordellii, C. celecrescens, C. histolyticum, C. sphenoides, C. subterminale, C. butyricum, C. septicum*) is described. The results could contribute to the empirical management of these infections in the trauma hospital.

Using the E-test methodology, the following resistance percentages were obtained: tetracycline 20%, clindamycin 28%, metronidazole 3%. All strains were sensitive to amoxicillin/clavulanate, ticarcillin/clavulanate and vancomycin. *C. tertium* showed the highest MICs to clindamycin (>256 µg/ml) and *C. perfrigens* to tetracycline (48 µg/ml).

LOW DOSE ARSENIC EXPOSURE AND CYSTIC FIBROSIS PROTEIN KNOCKDOWN ALTER THE EXPRESSION OF MICRORNAS IN THE INNATE IMMUNE RESPONSE TO *PSEUDOMONAS AERUGINOSA* INFECTION

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The innate immune system is essential for responding to different types of infections, injuries and that response can be altered by environmental toxicants, such as low doses of arsenic. Exposure to arsenic causes decreased overall innate immune function and reduces a host's ability to fight infections by opportunistic facultative anaerobic pathogens, like *Pseudomonas aeruginosa*, but the mechanisms are not well understood. Beyond environmental elements, genetic factors, such as the genetic disorder Cystic Fibrosis, further influence the innate immune response's dysregulation. The disorders is often characterized by a deficiency in the cystic fibrosis transmembrane conductance regulator (cftr) protein, which causes increased mucosal build-up, biofilm formation, decreased neutrophil function, and increased resistance to antibiotics with reduced ability to clear infections. We utilize the zebrafish (Danio rerio) as a model system to study how arsenic and knockdown of the cftr protein alter the innate immune response to infection by targeting its inhibitory mechanisms.

To further understand these mechanisms, our lab focuses on the highly conserved non-coding RNAs, microRNAs, responsible for regulating the protein-coding genes behind these mechanisms. The expression of microRNAs was characterized in 48 hours post-fertilization (hpf) control versus cftr morpholino embryos infected with *Pseudomonas aeruginosa* at 6 and 18 hours post-infection (hpi) following exposure to 0, 2, and 10 ppb arsenic. The highest number of differentially expressed microRNAs in response to infection at 6 and 18 hpi were in embryos exposed to 2ppb ppb arsenic with 81 and 87 microRNAs, respectively. Dre-Mir-199-P1, previously described to reduce neutrophil chemotaxis to sites of injury or infection, is normally upregulated in response to infection. At 6 hpi, it was upregulated with increasing concentrations of arsenic in cftr morphants, and an opposite effect occurred at 18 hpi. Our studies agree with preliminary findings regarding infection alone, while providing insight into the significant interactions of miR-199 between different treatment factors. These studies, combined with survival and bacterial burden studies, will provide new insight into the mechanisms of innate immune responses, and inform on the dysregulating effect of arsenic.

EIGHT-YEAR RETROSPECTIVE SURVEY OF ANAEROBIC BACTERIA ISOLATED FROM PROSTHETIC JOINT INFECTIONS IN A UNIVERSITY HOSPITAL

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Periprosthetic joint infection (PJI) is a serious complication of arthroplasty surgery. In PJI, both the diagnosis of the infection and the identification of the causative pathogen are crucial to optimize treatment outcomes.

In this study, we investigated range of organisms isolated from periprosthetic tissue samples (n=695) and synovial fluid samples (n=107), with an average of 4.2 samples per patient, submitted to Marmara University Pendik Training and Research Hospital Microbiology Laboratory for aerobic and anaerobic culture between 2014-2021. According to current guidelines, one positive culture was considered as significant for highly virulent organisms. For low-virulent organisms at least two positive culture (from separate samples) results were considered significant for infection (IDSA-2013).

A total of 802 culture results obtained from 189 patients were analyzed retrospectively. Among these 444 (55.4%) samples isolated from 139 patients were culture positive and 13,3% were polymicrobial. From those cultures *S. aureus* accounted for 42.1 % of all isolates, followed by coagulase negative *Staphylococcus* spp. (19,8%), Enterobacterales (13,1%), anaerobes (8,1%), non-fermenters (7,4%), *Enterococcus* spp. (6,3%), alfa and beta hemolytic *Streptococcus* spp. (2,3%), *Corynebacterium* spp. (1,4%), others (1,6%), respectively. Among 36 anaerobe organisms *Finegoldia magna* accounted for 33,4% of organisms, followed by *Veillonella* spp. (16,7%), *Bacteroides* spp. (13,9%), *Peptostreptococcus anaerobius* (11,1%), *Prevotella* spp. (5,5%), *Cutibacterium acnes* (8.3%), other gram positive bacili (11,1%). Twenty-four (66,6%) of anaerobe organisms were isolated from mixed infections

Current guidelines emphasized on the approach of commonly isolated organisms associated with PJI. Management of anaerobic organisms were not mentioned in the guidelines aside from *C. acnes*. For non-commonly isolated bacteria 4 to 6 weeks of pathogen-specific intravenous or highly bioavailable oral antimicrobial therapy is recommended.

In this study, we isolated highly virulent or low virulent anaerobic organisms. We believe that isolation and identification of anaerobes at the species level is necessary for a better understanding of the pathogenesis and for the implementation of an evidence-based treatment strategy in anaerobic PJI.

FENOLLARIA MASSILIENSIS ISOLATED FROM POLYMICROBIAL PROSTHETIC JOINT INFECTIONS OF A PATIENT WITH SACRAL SARCOMA

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The species *Fenollaria massiliensis* is a relatively unknown anaerobic Gram positive rod. *F. massiliensis*, isolated from an osteoarticular sample from a patient in France, was described in 2014. Eight clinical isolates of *F. massiliensis* were studied within the European Network for the Rapid Identification of Anaerobes (ENRIA) project. The strains were mostly encountered in clinical samples from the groin region. *F. massiliensis* is not represented in the VITEK MS database. Therefore, *F. massiliensis* can only be identified by 16S rDNA gene sequencing. We presented a case of *F. massiliensis* prosthetic joint infection in a 36-year-old patient with rare tumor affecting the sacrum.

Case Report: A patient, with a previous diagnosis of sacral sarcoma, radiotherapy, and chemotherapy, presented to our hospital with increasing in size of soft tissue mass. No clinically significant abnormality or neurologic deficit was detected. The patient underwent wide resection of the soft tissue of the pelvis and a vertebral pedicle screw rod system was used to provide ilio-lumbar stability. Three weeks after the surgical reconstructions, the patient was re-operated to open a loop colostomy due to a complication of faecal incontinence. The old incision line extending from the lumbar region to the sacrum was opened, the surrounding soft tissues of the implant were found to be highly infected and necrotic. Tissue specimens, obtained from infected area, were processed in terms of aerobic and anaerobic culture. Microbiological examination revealed polymicrobial infection involving six bacteria. Five organisms were identified by VITEK MS (bioMérieux) as *Bacteroides fragilis, Porphyromonas asaccharolytica, Actinomyces turicensis, Enterococcus faecalis* and *Corynebacterium striatum.* The sixth bacterium, for which MALDI-TOF MS was insufficient for identification, was identified by 16S rRNA gene sequencing as *F. massiliensis*. The patient was given long term imipenem therapy. The clinical condition improved considerably and regular follow-up of the patient was recommended.

In conclusion, the knowledge about *F. massiliensis* is limited, reporting of the similar cases will give insight in their clinical relevance.

PEPTONIPHILUS GROSSENSIS, VARIBACULUM CAMBRIENSE ISOLATED FROM POLYMICROBIAL ANAEROBIC BRAIN ABSCESS OF A MENTALLY RETARDED YOUNG PATIENT

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Brain abscess, which may occur secondary to otitis or mastoiditis, is a serious condition requiring early and accurate diagnosis, antibiotics, and surgical excision or drainage. The organisms most commonly isolated are anaerobic bacteria, aerobic and microaerophilic streptococci, Enterobacteriaceae, and *Staphylococcus aureus*. 16S rRNA-based amplification and sequencing significantly increased the number and variety of identified agents of brain abscesses. We presented the first case of human brain abscess, which consisted of *Peptoniphilus grossensis* and *Varibaculum cambriense.*

Case report: A 28-year-old mentally retarded patient with symptoms of nausea, vomiting, dizziness and unable to walk for the last 10 days was admitted to the emergency department of hospital. He had a history of recurrent otitis media attacks. Head CT and Diffusion MRI showed a hypodensity consistent with an abscess in the posterior fossa. He was subsequently transferred for emergent neurosurgical evaluation. Craiotomy, mastoidectomy cerebellar abscess drainage and duraplasty were performed.

The abscess material were inoculated onto proper media, incubated under aerobic and anaerobic atmospheres and incubation period for anaerobes extended to 14 days. We observed six different colony types on the primary plates out of which four colony types were identified by MALDI-TOF MS (VITEK MS; bioMérieux) with high confidence 99.9% as *Prevotella nigrescens. Actinomyces radingae, Parvimonas micra,* and *Anaerococcus vaginalis.* In case of two more colony types, found on the anaerobic culture plate after 4 days of incubation, the MALDI-TOF MS gave insufficient identification score. They were identified by 16S rRNA gene sequence analyzing as *Peptoniphilus grossensis* and *Varibaculum cambriense* with 99% nucleotide identity to the type strains in the GenBank database. The patient was given empirical moxifloxacin (1X400mg daily), he was discharged on the fifth day of hospitalization with oral moxifloxacin therapy for 6 weeks. The clinical condition improved considerably, and the patient was recommended to administer the same antibiotics an additional 2 weeks.

In Conclusion: Synergistic infectivity between strict anaerobes and other bacteria is a key factor in pathogenesis. The isolation and the defining of bacterial community composition associated with brain abscess can help shed light on the mechanisms of disease and impact of the bacteria.

POST DISCECTOMY SPINAL *CUTIBACTERIUM ACNES* AND *MYCOBACTERIUM TUBERCULOSIS* ABSCESS: A RARE COMPLICATION

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Postoperative infections are one of the most common complications of spine surgery. However, following a lumbar discectomy, a postoperative infection involving *Mycobacterium tuberculosis* as well as *Cutibacterium acnes* are extremely rare.

51-year-old male patient reported with a complaint of pain in lumbar region radiating to lower limbs associated with tingling and numbness for seven mounths and also had fever and weight loss complaints for one month. He had a history of lumbar discectomy four years ago. Magnetic Resonance Imaging of lumbar spine was done which revealed abscess at L4-L5 level. Laboratory analysis revealed systemic inflammation, with a plasma C-reactive protein level of 55,70 mg/L. Brucella Wright agglutination were negative. The patient underwent right L4 hemilaminectomy+L4-5 discectomy and spinal debridement. Four tissue and bone samples from operative site were sent for bacterial and mycobacterial culture which revealed no organism in Gram or Kinyoun staining. All of anaerobic bacterial cultures were found to be positive for *Cutibacterium acnes (C. acnes)*. Antibiotic susceptibility testing with the agar dilution method showed the organism to be sensitive to ampicillin, ampicillin-sulbactam, vancomycin, tetracycline, meropenem and clindamycin. The patient had been treated medically with six days of vancomycin and six weeks of levofloxacin therapy for *C. acnes*. On postoperative day 20, tissue culture grew *Mycobacterium tuberculosis* (*M. tuberculosis*). *M. tuberculosis* isolate was tested for susceptibility to isoniazid, rifampicin, ethambutol, and streptomycin and found susceptible. A four drug regimen of antituberculous treatment (Isoniazid, rifampicin, ethambutol, pyrazinamide) was commenced.

In the second month of antituberculosis treatment, he applied with the complaint of discharge in the operation area. The patient was re-operated because of the recurrence of vertebral abscess. Bacterial cultures were negative but tissue culture grew *M. tuberculosis.* After completing the antituberculosis treatment for one year, the patient's clinical condition improved, and the abscess gradually subsided.

Postoperative discitis is a relatively rare, but potentially serious complication of discectomy. The case reported here seems unique in the literature due to the co-existence of *M. tuberculosis* and *C. acnes*.

SIX-YEAR RETROSPECTIVE SURVEY OF INVASIVE *CUTIBACTERIUM* SPECIES INFECTIONS IN A UNIVERSITY HOSPITAL

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Cutibacterium species tend to be frequently reported as a contaminant, but studies have been published especially in the last 10 years showing that *Cutibacterium acnes* and other *Cutibacterium* species can cause serious, life-threatening invasive infections.

The first objective of this study was to evaluate the clinical presentations of *Cutibacterium* species – identified by MALDI-TOF MS (Vitek® MS) system – isolated from routine anaerobic culture from January 2015 to April 2021 at tertiary care hospital in Istanbul. The second objective was to determine the susceptibility of clinical isolates collected from different local or systemic infections to ampicillin, ampicillin-sulbactam, meropenem, tetracycline, vancomycin and clindamycin by agar dilution method.

The 252 samples were evaluated together with the information obtained from the hospital management system. Swab samples, cases where *Cutibacterium* species were only grown in a single set blood culture, cases without clinical evidence of infection at the site from which the sample originated were excluded. Therefore, a total of 45 isolates with accessible clinical information were included in the study.

The majority of isolates were isolated from adult (88.6%) and male (65.9%) patients. Most patients (75%) had a history of previous surgery. Foreign material was *in situ* at the infection site in 18,2%. The neurosurgery department provided most (37,7%-17/45) of isolates. Overall, 71,1% (n=32), 26,7% (n=12), 2,2% (n=1) of the isolates were identified as *C. acnes, C. avidum, C. granulosum,* respectively. All isolates of *C. acnes* and *C. granulosum* species were susceptible to the antibiotics tested. Clindamycin resistance was detected in 33,3% (4/12) of *C. avidum* isolates, and no resistance was found to other antibiotics.

In accordance with the literature in the world and in our country, the absence of resistance to ampicillin, ampicillin-sulbactam, meropenem and vancomycin is important in terms of guiding the empirical treatment of infection related *Cutibacterium* species.

Sunday, July 31 — *Clostridioides difficile*: Clinical

1315-1415 Poster Session II: *Clostridioides difficile:* Clinical

Posters will be presented in Poster Session II
Sunday, July 31 1315-1415.

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THE IMPACT OF ANTIBIOTICS, BACTERIAL CHALLENGE, AND IMMUNIZATION IN THE HAMSTER MODEL OF *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Clostridioides difficile, a Gram-positive spore-forming anaerobe, is the main cause of nosocomial infectious diarrhea in industrialized countries. A bivalent toxoid vaccine to prevent *C. difficile* infection (CDI) is in development. Disease is modeled preclinically using a hamster model of CDI: antibiotic treatment causes dysbiosis of the gut microbiome, followed by challenge with *C. difficile* spores. Prior studies have shown that hamsters vaccinated with toxoid-based vaccines are protected from CDI.

Our goal was to assess the effects of immunization, antibiotics, and bacterial challenge on the gut microbiome and toxin distribution in the CDI model. Genomic DNA was extracted from stool specimens, the V3-V4 region of the bacterial 16S rRNA gene was amplified from extracted DNA, libraries prepared/sequenced and data analyzed using Qiime2. Free toxin was detected with a cell cytotoxicity neutralization assay.

The Shannon diversity index of the gut microbiome was not statistically different between vaccinated and non-vaccinated groups. Antibiotic treatment altered alpha and beta diversity, reduced Firmicutes and increased Proteobacteria abundance. On day 1 following spore challenge of dysbiotic hamsters, the *C. difficile* component of the microbiome was not significantly different between vaccinated and non-vaccinated hamsters. Vaccinated animals survived for the study duration, while all non-vaccinated animals succumbed to CDI by day 2. Longitudinal recovery from dysbiosis was accompanied by a decrease in *C. difficile* abundance. At day 42 post challenge, the stool microbiome from most vaccinated hamsters had low or no detectable *C. difficile.* In separate studies, vaccinated animals subjected to a second antibiotic regimen and spore challenge at day 39/day 42 after the primary challenge did not present with clinical symptoms. Examination of cecum tissue revealed that toxin could be detected in vaccinated/challenged animals up to day 4 post challenge. In summary, vaccination protects hamsters from primary and secondary infections, despite the presence of *C. difficile* and toxin.

ANTIBIOTIC RESISTANCE PROFILE OF RT 027/176 *VS* OTHER *CLOSTRIDIOIDES DIFFICILE* ISOLATES IN SILESIA, SOUTHERN POLAND

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Clostridioides (Clostridium) difficile is an important health care-associated pathogen, responsible for a wide spectrum of diseases: from mild diarrhea to complications such as pseudomembranous colitis and toxic megacolon. The aim of this study was to analyze the antibiotic susceptibility profile of *C. difficile* isolates from fecal samples of patients hospitalized in 13 hospitals of the Silesian region of Poland in 2018-2019.

215 stool samples tested positive with C. diff Quick Check Complete were cultured anaerobically for *C. difficile.* Isolates were characterized, typed and tested for susceptibility to 11 antimicrobial agents by E-tests, according to EUCAST, 2020 criteria. Toxin A/B and binary toxin genes were detected by mPCR. The presence of the *ermB* gene (encoding resistance to macrolides, lincosamides and streptogramin B) was also studied. The history of previous fluoroquinolone treatment was documented in 30/215 patients with positive *C. difficile* culture. Among the studied *C. difficile* strains, 166 (77.2%) were classified as RT 027 and 6 (2.8%) as related RT 176; resistance to ciprofloxacin (96.7%), moxifloxacin (79.1%), imipenem (78.1%), penicillin (67%), and rifampicin (40.5%) was found. The *ermB* gene was detected in 79 (36.7%) strains. Multidrug resistance - MDR, defined as co-resistance to moxifloxacin, clindamycin, erythromycin, imipenem and rifampin was confirmed in 50 (23.3%) strains, predominantly belonging to RT 027 (94%).

In European countries, the incidence rates of CDI vary greatly depending on the region. In the Silesian region of southern Poland, the incidence per 100,000 people is higher than the average in Poland (31 vs 26.4, respectively). We described high prevalence of *C. difficile* belonging to RT 027, with increasing resistance to rifampicin and MDR, hence the need to enhance regional infection control on CDI and antibiotic stewardship in hospitals.

CONTEXT-SPECIFIC MODULATION OF *CLOSTRIDIOIDES DIFFICILE* VIRULENCE BY VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECIUM*

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Antibiotics can reduce microbial diversity giving opportunistic pathogens like *Clostridioides difficile* and Vancomycin-Resistant *Enterococcus faecium* (VRE) room to expand. VRE has frequently been reported as the most common co-colonizing species found in stool of *C. difficile* infection patients. Thus, defining interactions between the two species is crucial and may identify targets and strategies for non-invasive therapeutics. The initial objective of this study was to develop a co-culture biofilm model of VRE and *C. difficile*. For Gram-positive bacteria, this typically involves adding glucose to culture media to a concentration above 0.2% w/v. We found that the addition of glucose to culture media at concentrations above 0.4% results in complete inhibition of *C. difficile* vegetative growth in VRE conditioned medium. VRE is a lactic acid bacterium, and we confirmed that *C. difficile* growth is negatively correlated with glucose concentration and resulting lactic acid production. VRE conditioned medium with a pH below 5 fails to support *C. difficile* growth, consistent with published results. This growth suppression can be ameliorated by neutralizing acidified VRE conditioned medium with sodium hydroxide. Collectively, these data suggest that carbon source-dependent acidification of growth medium by VRE suppresses *C. difficile* growth. We are currently testing this hypothesis with a variety of carbon sources *in vitro*, some which are acidified by VRE and some which are not, and in a mouse model of co-infection. We are also testing a panel of enterococci and commensal Clostridia to determine if this is a generalizable mechanism that affects population structure in the gut. Carbon sources consumed by the host have the potential to shift the metabolite balance, organic acid abundance and pH of the gut to contribute to the outcome of this common co-infection.

MOLECULAR EPIDEMIOLOGY OF *CLOSTRIDIOIDES DIFFICILE* IN THE UNITED STATES, 2018

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In 2009, the Centers for Disease Control and Prevention (CDC) implemented *Clostridioides difficile* infection (CDI) surveillance through the Emerging Infections Program (EIP) to monitor the incidence and evolving epidemiology of CDI in the United States. Here we describe the molecular epidemiology of *C. difficile* isolates collected in the United States in 2018.

In 2018, CDI surveillance was conducted at 10 EIP sites (CA, CO, CT, GA, MD, MN, NM, NY, OR, TN). A convenience sample of clinical laboratories across EIP sites submitted *C. difficile*-positive stool specimens to the MN Department of Health Public Health Laboratory and Hines VA Hospital (IL) for culture. Isolates were forwarded to CDC and characterized by capillary-based PCR-ribotyping and PCR detection of *tcdA, tcdB, cdtA, cdtB,* and deletions in *tcdC.*

In 2018, 1,076 *C. difficile* isolates were submitted; the median number of isolates received from each site was 76.5 (range: 23-278). In total, 137 RTs were observed, with the majority of isolates harboring toxin genes *tcdA* and t*cdB* (95%) and a wildtype *tcdC* sequence (73%). Among 521 healthcare-associated isolates, RT 027 was the most prevalent (16%) and the most common RT detected at 5 sites (CT, GA, MD, NM, TN). Ribotype 106 was the most prevalent (16%) among 555 community-associated isolates and the leading RT detected at 8 sites (CA, CO, CT, GA, MD, MN, NY, OR). An overall increase in community-associated RT 106 was observed between 2012 and 2018 (9% vs 16%; $p=0.0007$), whereas RT 027 decreased from 2012 to 2018 among both healthcare-associated (21% vs 16%; $p=0.06$) and community-associated isolates (17% vs 4%; $p<0.0001$).

Our data demonstrate that the hypervirulent strain RT 027 with known antibiotic resistance has continued to decline in 2018. However, ongoing increases among other RTs, such as RT 106, highlight the evolving molecular epidemiology of *C. difficile* and the need for continued surveillance to monitor potential emerging strains.

CLOSTRIDIOIDES DIFFICILE INFECTION SURVEILLANCE IN WESTERN AUSTRALIA, 2015-2019

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In Western Australia (WA) since 2011, all isolates of *Clostridioides (Clostridium) difficile* from patients developing diarrhoea in hospital or presenting with diarrhoea to a hospital have been forwarded to PathWest Laboratory Medicine for typing, as part of the Healthcare Infection Surveillance Western Australia (HISWA) program. The objective of this study was to determine the molecular epidemiology of *C. difficile* infection (CDI) in children and adults identified in WA, 2015-2019. Deidentified HISWA CDI surveillance data were extracted for the period 2015-2019. Isolates from duplicate specimens taken within 7 days were included in the collection but excluded from the analysis. No demographic (except age) or clinical data was available. Of the 2791 isolates ribotyped during the study period, 136 (4.9%) were from paediatric patients with ages ranging from 1 to 17 years and the rest from adult patients. The most common ribotypes (RTs) isolated from paediatric patients were toxigenic (all sharing the same toxin profile, A+B+CDT-) RTs 020, 014 and 002 and non-toxigenic RT 010, whereas the most common RTs isolated from adult patients were toxigenic (A+B+CDT-) RTs 002, 014, 020 and 012. Interestingly, the hypervirulent strain RT 027 (A+B+CDT+) was isolated from a 72-year-old, and RT 078 was isolated from eight different adult patients, but no children. This is the first comprehensive review of the molecular epidemiology of CDI in an Australian state, over a 5-year period. The distribution of *C. difficile* RTs throughout WA appears similar to smaller studies in other states. Although there was no clinical information available, this data will form a baseline to evaluate changes in molecular epidemiology of CDI in WA.

CHARACTERIZATION OF SIMPLIFIED MICROBIAL COMMUNITIES THAT CAN INHIBIT *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Human fecal microbial transplantations (FMT) restore the homeostasis within the gut environment that resists *Clostridioide difficile* and are an effective treatment option in recurrent *C. difficile* infections (CDI). Safety concerns of FMTs arise due to indications that acute and chronic disease can be transferred and long-term effects on human health remain unknown, in addition, they have yet to be regulated by the Food and Drug Administration (FDA). Due to *C. difficile* being classified as an urgent threat by the Center for Disease Control and Prevention (CDC), there is an immediate need for an alternative and safe treatment option. We identified and characterized multiple defined and simple microbial communities originating from the human intestine that are aimed to prevent CDI and disease recurrence. Using a dilution/extinction approach coupled with rapid screening of resulting simplified communities in minibioreactor arrays, four simplified communities consisting of 15-30 members that reduce *C. difficile* invasion were identified. The identified communities clustered into distinct community types and shared less than four OTUs that were > 0.1% abundant. When further tested in a humanized microbiota mouse model, those communities significantly reduced the severity of initial CDI and limited susceptibility to disease relapse. Comparative analysis of fecal microbiomes from treated mice demonstrated that simplified communities accelerated recovery of indigenous bacteria and led to stable engraftment of some of the OTUs from simplified communities. Long-term safety and general health outcomes of two communities were assessed in germ-free C57BL/6J mice. No impact on host health in animals was observed for up to 12 months. To get a better understanding of key organisms that are required to resist the invasion to the pathogen, deep sequencing and metabolomic analysis of the simplified communities is currently being conducted. Individual strains have been isolated and will be characterized with a final goal to reconstitute the communities one strain at a time.

A PREDICTIVE MODEL TO IDENTIFY COMPLICATED *CLOSTRIDIODES DIFFICILE* INFECTION

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Background: *Clostridioides difficile* infection (CDI) is a leading cause of health-care-associated infections and may result in organ dysfunction, colectomy, and death. We recently showed that published risk scores to predict severe complications from CDI demonstrate poor performance upon external validation. We hypothesized that building and validating a model using geographically and temporally distinct cohorts would more accurately identify patients at risk for complicated CDI.

Methods: We conducted a retrospective cohort study of adult subjects diagnosed with CDI at three centers in the US. After randomly partitioning the data into training/validation sets, we developed and compared three machine learning algorithms (Lasso regression, random forest, stacked ensemble models) with 10-fold cross-validation that used structured EHR data collected within 48 hours of CDI diagnosis to predict disease-related complications from CDI (intensive care unit admission, colectomy, or death attributable to CDI within 30 days of diagnosis). Model performance was assessed using area under the receiver operator characteristic curve (AUC).

Results: A total of 3,762 patients with CDI were included of which 218 (5.8%) had disease-related complications. All models, including Lasso regression, random forest, and stacked ensemble models, performed well in the validation set with AUC ranging between 0.89-0.9. Variables of importance were similar across models, including albumin, bicarbonate, change in creatinine, systolic blood pressure, non-CDI-related ICU admission, and concomitant non-CDI antibiotics within 30 days of CDI diagnosis. Sensitivity analyses indicated that model performance was robust even when selecting different derivation and validation cohorts based on sites and different CDI testing approaches (e.g. PCR- vs. toxin-based diagnosis).

Conclusion: Using a large heterogeneous population of patients, we have developed and validated a prediction model based on structured EHR data that accurately estimates risk for complications from CDI.

COMPARISON OF FIDAXOMICIN AND ORAL VANCOMYCIN FOR THE TREATMENT OF *CLOSTRIDIOIDES DIFFICILE* INFECTION IN HOSPITALIZED PATIENTS RECEIVING CONCOMITANT ANTIBIOTICS FOR THE TREATMENT OF CONCURRENT INFECTIONS

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Background: Even after recovery from *Clostridioides difficile* infection, recurrent CDI (rCDI) occurs frequently, more so with concomitant antibiotic (CA) use for treatment of a concurrent infection. Fidaxomicin versus vancomycin for CDI has a similar rate of cure but a lower recurrence risk, but their relative performance is unknown in those receiving CA.

Methods: We conducted a randomized, controlled, open-label phase IV trial at the University of Michigan and St. Joseph Mercy hospitals. After informed consent, we enrolled hospitalized patients ≥18 years old with a positive test for toxigenic *C. difficile*, >3 unformed stools/24 hours, and ≥1 qualifying CA with planned duration ≥5 days. Selected exclusion criteria were complicated CDI, treatment for >24 hours prior to enrollment, concomitant laxatives, bowel ostomy, and planned CA use >12 weeks. Clinical cure was defined as resolution of diarrhea for 2 consecutive days maintained for 2 days after end of treatment; and rCDI was recurrent diarrhea with positive testing within 30 days of initial treatment. Patients were randomized (stratified by ICU status) to fidaxomicin 200 mg twice daily or vancomycin 125 mg orally four times daily for 10 days. If CA were continued > 10 days, the study drug was continued until the CA ended. Bivariable statistics included t-tests and chi-squared tests.

Results: After screening 5101 patients for eligibility, 144 were randomized. Baseline characteristics were similar between groups. Most patients were younger than age 65, on PPIs, and not in the ICU. In the intention-to-treat population, clinical cure was higher for fidaxomicin (73% vs. 62.9%, *P* =.195) but rCDI (3.3% vs 4.0%; *P* >.99) was similar.

Conclusions: In this study of patients with CDI receiving CA, a non-significant but numerically higher proportion were cured with fidaxomicin vs. vancomycin. Recurrence was lower than in both arms thanprevious studies that did not extend duration of CDI treatment during CA. Future studies are needed to ascertain if clinical cure is higher with fidaxomicin than vancomycin during CA exposure, and whether extending the duration of CDI treatment reduces recurrence.

MICROBIOME RESPONSES TO FECAL MICROBIOTA TRANSPLANTATION IN DOMESTIC CATS

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Purpose: To evaluate FMT's ability to improve feline CE signs, examining corresponding microbiome shifts.

There is growing interest in the application of FMTs in small animal medicine, but there are few published studies testing their effectiveness. Here we examine the microbiome changes observed in sixty-eight domestic cats undergoing FMT capsule treatment for chronic digestive issues. FMT capsules contained screened fecal material from 8 healthy pet cats, and each treatment consisted of 50 capsules given orally over ~25 days. Fecal samples were collected pre-FMT and 2 weeks post FMT treatment, and data on the cat's health was documented. The bacterial 16S rRNA gene was sequenced for fecal samples and data from 82 cats with no disease diagnoses comprised a healthy reference. We found that 78% of cats reported improvement in their clinical signs (Responders), and 22% exhibited no change or a worsening of their clinical signs (Non-responders). Changes in bacterial relative abundances in pre and post-FMT samples indicated that Responders exhibited increased Peptococcus ($P=0.001$), decreased Prevotella 9 ($P=0.03$), and decreased Sutterella ($P=0.03$) compared to Non-responders. Escherichia abundances increased significantly post-treatment ($P=0.039$) in Non-responders, and remained unchanged in Responders. Lastly, Responders' microbiomes grew more similar to healthy cat microbiomes as a result of FMT treatment, while Non-responders grew less similar to the healthy reference ($P=0.0486$). FMT recipients with a prior IBD diagnosis ($P<0.0001$), and those reported to only have chronic diarrhea also exhibited increases in similarity to the healthy reference set ($P<0.0001$) compared to other cats.

Conclusion: Clinical signs improved after oral FMT treatment. Gut microbiome of Responders shifted positively towards the healthy reference set. Escherichia is an underappreciated response indicator to FMT success.

DEFINING THE IMPACT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS DURING *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Clostridioides difficile is the most commonly reported nosocomial pathogen and is an urgent public health threat. This Gram-positive, spore-forming anaerobic bacterium colonizes the colon, causing a wide range of symptoms varying in severity from mild diarrhea to toxic megacolon and/or death. The factors responsible for this broad spectrum of disease are largely unknown, but likely include host, microbiota, and environmental factors. Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most used pharmaceutical drugs in the world and function through targeting cyclooxygenases (COX) enzymes and blocking the production of prostaglandins. We recently demonstrated that NSAIDs worsen the course of *C. difficile* infection (CDI). Mice infected with *C. difficile* and pre-treated with Indomethacin, a non-selective COX inhibitor, showed dramatic exacerbation of disease and disruption of the epithelial cell barrier. Additionally, we were able to show that prostaglandin E2 (PGE2), one of the most abundant COX products in the gut, protects against severe CDI. Mice infected with *C. difficile* and treated with misoprostol, an FDA-approved PGE2 analog, showed increased survival—a phenomenon driven by maintenance of tight junction integrity and recovery of the epithelial cell barrier. To dissect the mechanism of NSAIDs and prostaglandin-mediated effects during CDI, I have developed an *in vivo* and *in vitro* system using mice and Caco-2 intestinal epithelial cells. First, I have shown that during NSAID treatment in Caco-2's followed by *C. difficile* intoxication, there is an increase in epithelial cell death. Moreover, NSAIDs are able to decrease epithelial cell permeability in the presence of *C. difficile* toxins. Despite their COX inhibiting role, NSAIDs are agents that can uncouple mitochondrial respiration. My preliminary data demonstrate that NSAIDs uncouple mitochondrial functions during CDI leading to an increase in epithelial cell death. Moreover, I have shown that NSAIDs are able to sensitize mitochondria in epithelial cells prior to *C. difficile* infection. In a mouse model of infection, mitochondrial uncouplers phenocopy NSAIDs and both lead to an increase in mortality, weight loss, and epithelial cell damage in mice. I hypothesize that during CDI, NSAIDs uncouple mitochondrial functions leading to an increase in epithelial cell death and permeability. Impairment of epithelial cell barrier functions allows for microbes to translocate into the lamina propria leading to a robust pro-inflammatory response.

ILLUMINATED *CLOSTRIDIOIDES DIFFICILE* SPORES IMPROVES SURFACE DISINFECTANT PROCESSES, HELPING REDUCE HOSPITAL-ACQUIRED *C. DIFFICILE* INFECTIONS

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LIV Process was developed to revolutionize the deadly impact *Clostridioides difficile* (*C. difficile*) has on the world. LIV (Light, Identify, Verify) Process created a proprietary infection control solution that instantly illuminates *C. difficile* spores on any surface–large or small. Three people die every hour from a *C. difficile* infection (CDI), and LIV Process recognized the significant shortcomings surrounding detection of deadly *C. difficile* spores on surfaces.

LIV Process' breakthrough surface detection solution is the only product on the market specific for *C. difficile* spore identification. Prior to LIV Process, it was not possible to see these deadly blind spots on surfaces, thus creating great risks for patients, healthcare specialists, cleaning staff, and visitors.

In advance of its 2023 launch, LIV Process conducted a clinical study in a large New Jersey hospital following the below parameters:

- Analyzed 22 surfaces that were identified as "high-touch" hospital room surfaces
- LIV Process was applied after Environmental Services (EVS) staff disinfected a discharged patient's room according to the hospital's current disinfection protocols
- LIV Process personnel was responsible for LIV Process application and data acquisition

Study highlights include:

- Applied LIV Process to 114 rooms, analyzing >2,000 surfaces
- Of all the surfaces examined, 23% were identified as contaminated and 2% verified as still contaminated after a second round of disinfection
- >50% surfaces per room were identified to be contaminated during the first weeks of the study, versus <15% at the end
- An unexpected pause in study operations due to COVID spikes and the winter holidays caused contaminated surfaces per room to increase by 10-15%
- 45% of high-touch surfaces had a >20% chance of being contaminated, with three surfaces (bedrails, glovebox, and footboard) having >50% chance of being contaminated
- Of the total number of bed surfaces LIV Process assessed, 56% were contaminated and 5% were verified to still be contaminated during the verification assessment which is supported by recent independent research correlating contaminated hospital beds to increased risks of CDIs

This presentation will provide an overview of LIV Process' surface detection system, a detailed description of our clinical study design and outcomes and highlight the impact that the LIV Process can have on hospital-acquired CDIs.

July 28-31

Sunday, July 31 *Clostridioides difficile*: Pathogenesis

1315-1415 Poster Session II: *Clostridioides difficile:* Pathogenesis

Posters will be presented in Poster Session II
Sunday, July 31 1315-1415.

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Sunday, July 31 *Clostridioides difficile*: Pathogenesis

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Posters will be presented in Poster Session II
Sunday, July 31 1315-1415.

DEVELOPMENT OF HIGH-QUALITY REFERENCE GENOME SEQUENCES FOR DIVERSE *CLOSTRIDIOIDES DIFFICILE* RIBOTYPES

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The Emerging Infections Program (EIP) at the Centers for Disease Control and Prevention (CDC) performs *Clostridioides difficile* infection surveillance, including the characterization of isolates submitted from 10 sites across the United States. *C. difficile* have diverse genomes containing various mobile elements, and short- and long-read whole genome sequencing (WGS) is required to generate accurate assemblies. Most publicly available complete genome sequences correspond to the epidemic ribotype (RT) 027 lineage. We selected 10 *C. difficile* isolates representing the top 10 RTs from the 2016 EIP surveillance year for WGS to generate high-quality reference genome sequences and identify extrachromosomal elements.

High molecular weight genomic DNA was extracted from 10 isolates (RTs 027, 056, 002, 019, 078, 015, 054, 106, 014, and 020), and long-read sequencing data were generated using the PacBio Sequel II System. Short-read WGS (Illumina MiSeq) data were used to generate hybrid assemblies (Unicycler 0.4.8 and SPAdes 3.14.0) and visualized with Bandage. BLASTN was used to characterize plasmid and bacteriophage sequences.

Genome sizes ranged from 4.0-4.4 Mb and 6 of 10 genomes were closed, with the 4 unclosed genomes averaging 16.5 contigs. Five isolates harbored plasmids (4.7-130 kb) that shared similarity with pCDBI1-, pCD630-, and pCD-WTSI-like plasmids. Circular prophages were identified in 2 isolates, including sequences with similarity to phiCD38-2 (41 kb, 100% coverage, 99.97% identity; RT019) and phi6356 (38 kb, 71% coverage and 93.5% identity; RT002).

There is limited knowledge about the roles of mobile elements in *C. difficile* pathogenesis. High-quality reference sequences from diverse RTs are a critical first step to facilitate characterization of bacteriophages and plasmids in *C. difficile*. These high-quality reference sequences are publicly available on NCBI, and isolates can be requested through the CDC & FDA Antibiotic Resistance Isolate Bank.

IBEZAPOLSTAT IS EFFECTIVE *IN VITRO* AGAINST HIGH INOCULUM *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Introduction. The bacterial load in patients with *Clostridioides difficile* infection (CDI) can be as high as 10^8 CFU/ gram stool. Despite this, antibiotics in the drug development pipeline rarely test *in vitro* activity at high inoculums. The purpose of this study was to assess the *in vitro* killing activity of ibezapolstat, a Gram-positive selective antibiotic currently in phase 2 studies *vs*. available comparators at high initial inoculums.

Methods. C. *difficile* strain R20291 was prepared by inoculating 20mL brain heart infusion-supplemented with bile salts broth with a single colony and incubated overnight at 37°C in an anaerobic chamber. Cultures were diluted to standard (10^5-6 CFU/mL) or high (10^7-8 CFU/mL) inoculum and ibezapolstat, vancomycin and fidaxomicin were added at concentrations of 4-64 µg/mL plus controls. Total cell and spore counts were measured at 24h and 48h. Toxin A and B concentrations were measured by EIA.

Results: At standard inoculum, all antibiotics displayed potent bactericidal activity (3-log CFU/mL decrease) at 24h and 48h. Spores were only detectable in vancomycin experiments at 48h. At high inoculums, bactericidal activity was not observed at 24h for any antibiotic. At 48h, both ibezapolstat and fidaxomicin displayed increased potency compared to vancomycin including decreased total and spore CFU/mL counts. Toxin A and B measurements correlated with starting inoculum, measurement time, and total CFU/mL counts.

Conclusion: Ibezapolstat displayed favorable killing kinetics compared to currently approved CDI-directed antibiotics at standard and high inoculums. These results support the continuing development of this first-in-class antibiotic.

THE NOVEL ADJUVANT, ADA-1, RESTORES AGE-ASSOCIATED DEFECTS IN THE ADAPTIVE IMMUNE RESPONSE TO *CLOSTRIDIOIDES DIFFICILE* INFECTION AND VACCINATION IN AN AGING MOUSE MODEL

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C. difficile is the leading cause of healthcare-associated infection in the United States. The elderly (≥65) have the greatest risk of *C. difficile* infection (CDI), severe morbidity, and mortality due to CDI. While previous studies have shown that strong humoral responses against *C. difficile* Toxin A/B (TcdA/B) during primary CDI or vaccination are protective against severe disease and recurrence, the elderly have impaired humoral responses against TcdA/B during primary CDI and vaccination. Using an aging mouse model of CDI, we show that there are significantly greater morbidity and mortality in aged mice during primary CDI compared to young adult mice, mirroring what is seen in elderly CDI patients. Additionally, in response to both primary CDI and vaccination with TcdA/B-encoding DNA, aged mice have significantly lower anti-TcdA/B antibody titers. We further show that this impaired humoral response is the result of poor Tfh and B cell responses following infection and vaccination as aged mice have a significantly lower frequency of Tfh cells and number of anti-TcdA/B B cells when compared to young adult mice. While these age-associated defects in the humoral response allow for increased vulnerability to CDI, we also show that these defects can be restored with the addition of the molecular adjuvant ADA-1 during vaccination of aged mice with TcdA/B-encoding DNA. With the addition of ADA-1, the anti-TcdA/B antibody response in aged mice is restored to levels seen in young adult mice. ADA-1 also boosts the frequency of Tfh cells in aged mice following vaccination, suggesting that ADA-1 helps restore the development of the aged germinal center reaction following vaccination. As such, this work highlights both the need for the development of a *C. difficile* vaccine with the elderly in mind and presents ADA-1 as a solution to overcoming age-associated defects in the vaccine response of the elderly.

CLOSTRIDIOIDES DIFFICILE 630 ENCODES A COMPLETE NON-CONTIGUOUS AGR1 SYSTEM WHICH POSITIVELY IMPACTS SPORULATION

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Endospore formation is initiated by phosphorylation of the conserved transcriptional regulator, Spo0A. Multiple kinases activate Spo0A in other spore-forming organisms; however, these factors are not conserved in *Clostridioides difficile*. Three predicted histidine kinases repress *C. difficile* sporulation by negatively impacting Spo0A activity, yet no factor that directly phosphorylates Spo0A has been identified. Here, we investigated the impact of an additional orphan histidine kinase, CD0576, on *C. difficile* 630 sporulation. CRISPR*i* knockdown and deletion of *CD0576* resulted in significantly reduced sporulation frequency but did not impact toxin or motility gene expression. Interestingly, the HTPase domain of CD0576 shares homology with AgrC, the conserved histidine kinase of the Gram-positive accessory gene regulator (Agr) system, and VirS, which is the likely histidine kinase in the *Clostridium perfringens* Agr system. Almost all sequenced *C. difficile* strains encode a partial *agr* locus, comprised of the autoinducing peptide (*agrD1*) and a protease (*agrB1*). Previous work showed that the *agrB1D1* locus in *C. difficile* 630 positively impacts sporulation, but a cognate AgrC1 histidine kinase and AgrA1 response regulator were not identified. Further, a previously studied VirR-like orphan response regulator, RgaR, was shown to directly bind and activate *agrB1D1* transcription. We hypothesized that CD0576 and RgaR are the cognate AgrC1 and AgrA1 in *C. difficile* 630. To test this, we performed CRISPR*i* knockdown and deletion of *rgaR*, which also resulted in significantly decreased sporulation. We also found that *agrB1D1* transcript levels were similarly decreased in the *CD0576* and *rgaR* mutants, as well as transcript levels of two additional direct RgaR targets, *CD0587* and *CD2098*. These results suggest that CD0576 and RgaR function as a two-component system to regulate the primary *agr* locus in *C. difficile*. Our data suggests that CD0576, RgaR, and *agrB1D1* comprise the complete Agr1 system in *C. difficile* 630 that positively influences *C. difficile* sporulation through an undiscovered mechanism.

CLOSTRIDIOIDES DIFFICILE TOXIN-INDUCED MIF RELEASE IS ASSOCIATED WITH INTESTINAL EPITHELIAL CELL DEATH

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Clostridioides difficile (*C. difficile*) is a leading cause of nosocomial infections in the US. The main virulence factors of *C. difficile* are its secreted toxins which cause intestinal epithelial cell (IEC) injury resulting in a robust inflammatory response. Although current therapies primarily target the pathogen, multiple lines of evidence suggest that the degree of host inflammatory response is a better indicator of *C. difficile* infection (CDI) outcomes than pathogen burden. We have previously identified that high levels of the pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF), is deleterious in CDI. Blocking MIF in mice with CDI resulted in lower mortality and clinical disease, without effects on pathogen load. Therefore, elucidating the pathogen-associated drivers of MIF expression, identifying the main cells that produce MIF and the mechanisms of its release during CDI can determine novel "host-directed" targets for CDI treatment.

IECs are the first cells to interact with *C. difficile* toxins. Therefore, we examined MIF production from human (Caco-2) and mouse (CMT-93) IEC lines exposed to *C. difficile* toxins (TcdA and TcdB). Our data reveal that at 48 hrs after TcdA/TcdB exposure, MIF levels increase both intracellularly and extracellularly. The increase in MIF release inversely correlated to cell viability. To start examining the effects of toxins on immune cells, we utilized mouse splenocytes, peripheral blood mononuclear cells (PBMCs), and granulocytes. Our preliminary data suggests that TcdA/TcdB-treated mouse splenocytes, PBMCs, and granulocytes do not release extracellular MIF *in vitro* at 3 hrs, 24 hrs, and 48 hrs in response to toxin exposure. Together, our data suggest that IECs are an important producer of MIF and that *C. difficile* toxins are sufficient to induce its expression and extracellular release. Our future studies are focused on examining MIF induction and its release pathway using human intestinal organoids and *in vivo* animal models of CDI.

D-PROLINE REDUCTASE UNDERLIES PROLINE-DEPENDENT GROWTH OF *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile is a nosocomial bacterial pathogen that colonizes the gut and causes diarrhea, colitis, and severe inflammation through the activity of its exotoxins. Recently, *C. difficile* has been shown to use toxin-mediated inflammation to promote host collagen degradation, which releases several amino acids into the environment. Amino acids act as electron donors and acceptors in Stickland metabolism, an anaerobic process involving redox reactions between pairs of amino acids. Proline, glycine, and hydroxyproline are the three main constituents of collagen and are assumed to act as electron acceptors, but their exact effects on the growth and physiology of *C. difficile* are still unclear. Using three standard culture media (BHIS, TY, and CDMM) supplemented with proline, glycine, or hydroxyproline, we grew *C. difficile* strains R20291, JIR8094, and a panel of mutants unable to express the Stickland selenoenzymes D-proline reductase and glycine reductase. These mutants were deficient in either D-proline reductase (*prdB*, *prdR*), glycine reductase (*grdA*), or both enzymes due to the removal of selenophosphate synthetase (*selD*). Growth yields of the wild-type strains were higher in rich media (BHIS and TY) supplemented with proline and hydroxyproline but not glycine; moreover, we observed that proline-stimulated growth yields required D-proline reductase, whereas hydroxyproline-stimulated growth yields proceeded independently of the selenoenzyme. While assumed to be a proline auxotroph, *C. difficile* could surprisingly grow in a defined minimal medium (CDMM) without proline but only when D-proline reductase was absent. We believe the mere presence of this enzyme ultimately determines the organism's strict dependence on proline and likely defines the bioenergetic priorities for thriving in the host. Finally, we demonstrated that proline, and hydroxyproline could reduce toxin production but not in cells without selenoproteins. Our studies emphasize the importance of D-proline reductase in *C. difficile* physiology, particularly in the role of scavenging collagen-derived nutrients.

ELUCIDATION OF THE MECHANISM AND IMPACT OF THE MICROBIOME ON *CLOSTRIDIOIDES DIFFICILE* INDUCED COLONIC TUMORIGENESIS

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Human sporadic colorectal cancer (CRC) persists as a global public health burden as the second most frequently diagnosed cancer in both women and men and third in cancer-related deaths worldwide. Mucus-invasive bacterial biofilms are identified on the colon mucosa of approximately half of CRC patients. Recent work within the Sears laboratory identified the homogenates of human biofilm-positive colon mucosa prepared from 5 tumor patients undergoing screening colonoscopy can induce tumor formation in mouse colon tumor models. Further investigation of the bacterial composition of selected individual patient homogenates yielded isolation of 29 individual bacterial species including, unexpectedly, *Clostridioides difficile*. Strikingly, preliminary data now indicate that tumor formation is driven by the presence of the *C. difficile* strains. Our research focuses on the investigation of the bacterial genetic and molecular factors pertaining to *C. difficile*-induced tumor formation. We hypothesize that genetically-unique strains of *C. difficile* are influenced by the microbiota to induce environmental responses that yield persistent mucosal colonization and pro-tumorigenic immune and colon epithelial cell signaling. We have identified specific members of the biofilm human biofilm-positive colon mucosa that alter *C. difficile* behavior *in vitro* including modification of *C. difficile* Toxin A/B production and growth characteristics that may be essential for tumorigenesis. Among the 28 mucosally derived isolates, only 6 appear critical to modifying *C. difficile* biology where communities of 2 of these isolates strongly impact *C. difficile* pathogenesis. RNA-seq, metabolite and *in vivo* mechanistic studies are in progress. Our results suggest specific microbial interactions that may foster persistent *C. difficile* colonization with tumorigenic potential. This knowledge may be utilized with the aim of informing and improving patient health through new screening modalities and treatment regimes.

CLOSTRIDIOIDES DIFFICILE STRAIN CHARACTERIZATION: WHOLE GENOME MULTILOCUS SEQUENCE TYPING FROM NEXT GENERATION NUCLEOTIDE SEQUENCE PROVIDES GREATER GRANULARITY THAN PCR RIBOTYPE

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Clostridioides difficile is a Gram-positive bacterium that causes infectious diarrhea in healthcare and community settings. There is no approved vaccine to prevent initial or recurrent *C. difficile* infection. In addition to the design and assessment of vaccine effectiveness, bacterial strain typing is necessary for an understanding of disease outbreaks and dissemination of infection. PCR ribotyping, amplification of the intergenic spacer region between 16S and 23S rRNA genes, has been the method of choice for *C. difficile* strain typing. Multiple ribosomal alleles within the *C. difficile* genome give rise to several PCR fragments which, after size fractionation, provide a profile that is used to assign a ribotype (RT). Constraints on PCR ribotyping range from methodological differences for optimal size fractionation of PCR products to the absence of a panel of reference strains that are shared by global testing labs. A complex profile of poorly resolved fragments in the absence of reference standards can lead to incorrect RT assignments or force the assignment of an ambiguous hybrid RT (e.g, RT014/020). As a consequence, strain characterization across global collections based upon RT assignments can be challenging. Advances in instrumentation and sequence analysis pipelines have enabled routine access to bacterial whole genome sequence (WGS), data which can provide more detailed epidemiological information than classical PCR ribotyping. Whole Genome Multilocus Sequence Typing (wgMLST) uses specific genetic markers extracted from WGS to provide an unbiased approach to measure strain relatedness. In this study, the WGS and RT of a collection of *C. difficile* clinical isolates was determined. While generally consistent with strain characterization based on RT, detailed wgMLST analysis of WGS data provides additional granularity and the ability to differentiate strains with closely related and/or hybrid RT assignments.

THE ANCHORING OF THE POLYSACCHARIDE II IS ESSENTIAL FOR *CLOSTRIDIOIDES DIFFICILE* SURVIVAL

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While toxins are described to be the major virulence factors in *C. difficile* infections, there is an increasing interest in the role of non-toxin factors in pathogenesis and virulence. In this study, we investigated the role of the two *lcp* genes, supposed to be non-redundant and known to be involved in the synthesis of the polysaccharide II by anchoring filaments to the peptidoglycan [1]. In this study, we constructed single and double mutants of *lcp* genes. Surprisingly, we were unsuccessful to obtain the double mutant, whereas the two single mutants were easily obtained by an allelic exchange technique. This suggests that lcp genes have indeed redundant functions. To delete both *lcp* genes, we developed a conditional lethal mutant technique. The first step was to construct a strain containing a second copy of *lcpB* in a chosen region of the chromosome, expressed under the control of an anhydrotetracycline inducible promotor, pTet. Then, we replaced the ORFs by a resistance cassette in the native locus and so deleted both *lcpA* and *lcpB*. In this conditional mutant, we were able to modulate the expression of *lcpB*. Thanks to this tool and the production of highly specific anti-polysaccharide II antibodies, we highlighted the essentiality of the polysaccharide II anchoring. Using immunofluorescence microscopy, we showed that in single mutants, the polysaccharide II layer is abnormal in comparison to the wild-type strain. It is discontinued and holed in the *lcpA* mutant but smooth and inhomogeneous in the *lcpB* mutant. In the double mutant with low *lcpB* expression, we observed ellipsoid cells. Complementation with *lcpA* or *lcpB* restores the rod-shape morphology and the normal abundance of polysaccharide II. Additional results show a defect of the S-layer anchoring in the conditional mutant strain. In conclusion, our results show the critical role of polysaccharide II anchoring in growth, elongation, and correct surface set-up of *C. difficile*. Our technique provides new opportunities to study essential genes in *C. difficile.*

[1] M. Chu et al., PLoS Pathog., vol. 12, no 10, oct. 2016.

INTERSECTION BETWEEN IRON ACQUISITION AND PATHOGENESIS IN *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile, a gram positive obligate anaerobe, produces toxins *TcdA* and *B* that damage the gastrointestinal tract and cause diarrhea. Ferrous iron acquisition is critical for survival and pathogenesis of various gastrointestinal pathogens. *C. difficile* encodes three homologs of the ferrous iron transporter FeoB, of which FeoB1 is thought to be the main transporter of ferrous iron. Deletion of *feoB1* was shown to cause an accumulation of pyruvate, which is thought to inhibit toxin production. To further investigate FeoB1's role in pathogenesis, we constructed a *feoB1* deletion mutant R20291. The mutant showed significantly reduced levels of TcdA and TcdB, when compared to the WT and complemented mutant, and was avirulent in mice. We sought to understand transcriptome changes resulting from loss of *feoB1*. To compare the mutant with the WT, RNAseq was performed on samples collected at early log, mid-log and stationary phase. This identified that there were >300 differentially expressed genes. Iron acquisition genes were upregulated, including the genes for *feoA1*, *feoA2* and *feoB2*. In addition to upregulation of genes involved in ferrous iron acquisition, genes for zinc and cobalt transport and flavodoxin were upregulated. Furthermore, genes involved in sugar fermentation e.g., succinate to butanoate were upregulated. Conversely, downregulated genes included ferric iron transporters and the iron storage protein bacterioferritin. Consistent with phenotypic data showing that the mutant was less virulent, genes in the pathogenicity locus were downregulated (i.e., *tcdA*, *tcdB*, *tcdE*, and *tcdR*). These findings clearly establish a link between iron availability and toxin synthesis, warranting future studies to determine the mechanism by which lack of FeoB1 causes a virulence and whether FeoB1 could be a therapeutic target.

INVESTINGATING THE EFFECTS OF ADAPTIVE IMMUNE SYSTEM DEFICIENCY ON *CLOSTRIDIOIDES DIFFICILE* BIOLOGY

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Clostridioides difficile is the leading cause of nosocomial infectious diarrhea in the United States. Susceptibility to infection is driven by a disruption of the microbiota, most commonly due to antibiotic treatment. Symptoms caused by *C. difficile* infection range from mild diarrhea to potentially life-threatening forms like pseudomembranous colitis and toxic megacolon. While the host immune factors that determine the degree of epithelial damage and disease severity have been extensively studied, how the host immune response affects the biology of the pathogen in the intestine is less well understood. Previous work from our group demonstrated that T and B cell deficient $Rag1^{-/-}$ mice exhibited elevated expression of proinflammatory genes compared to infected littermate Rag1 heterozygous control mice ($Rag1^{HET}$) and were refractory to fecal microbiota transplant (FMT)-mediated resolution of *C. difficile.* We sought to determine how the host's adaptive immune response alters the transcriptional activity of *C. difficile* during persistent infection. To study this, we compared *C. difficile* gene expression in the cecum of infected $Rag1^{-/-}$ mice and $Rag1^{HET}$ control mice at 21 days post-infection. Sequencing of RNA isolated from the intestinal contents revealed differences in genes involved in metabolism and nutrient transport between the mouse genotypes. These preliminary results suggest that the adaptive immune system alters the nutrient availability within the intestine and affects *C. difficile* metabolism.

CLOSTRIDIOIDES DIFFICILE SMALL ACID-SOLUBLE PROTEINS PERFORM A NOVEL ROLE IN SPORULATION

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Clostridioides difficile transmits from host-to-host by forming dormant endospores that persist in the environment. Spores are metabolically dormant forms of bacteria that survive desiccation, extreme temperatures, and chemical and UV exposure. The overall structure of endospores is conserved among spore-forming bacteria. Inside, the core contains DNA, RNA, dipicolinic acid, and proteins, such as the small acid-soluble proteins (SASPs). In *B. subtilis,* the primary SASPs, SspA and SspB, protect the DNA from UV damage by coating the DNA and altering the DNA conformation to discourage cis-syn thymine dimer formation. *C. difficile* encodes *sspA* and *sspB* orthologues. Though the *C. difficile* SASPs functioned as predicted in UV resistance, we surprisingly found that the combined deletion of *sspA* and *sspB* prevented spore formation. This suggests a possible regulatory role of SspA and SspB during sporulation, a previously unreported SASP function. Using an ethyl methanesulfonate (EMS) mutagenesis selection strategy, we identified mutations in CDR20291_0714 that suppressed the asporogenous phenotype. CDR20291_0714 is a predicted peptidase that is homologous to *B. subtilis* SpoIVB, a protein involved in the signaling cascade for sporulation. Investigations are ongoing to determine the pathway in which SspA, SspB, and *CDR20291_0714* interact to influence sporulation in *C. difficile.*

DEFINING TOXIN-DEPENDENT MECHANISMS OF DIARRHEA DURING *CLOSTRIDIOIDES DIFFICILE INFECTION*

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Clostridioides difficile infection (CDI) is the leading cause of hospital-acquired diarrhea in the USA. Symptoms can range from severe diarrhea to fulminant colitis, toxic megacolon, and death. CDI is mediated by two protein toxins, TcdA and TcdB, that are produced by *C. difficile* in the colon during pathogenesis. Following receptor mediated endocytosis of the toxins, the glucosyltransferase (GT) domain is translocated and released into the cytosol. The GT domain inactivates Rho family GTPases, causing cytoskeletal rearrangements, cytokine release, and apoptosis. Mechanisms of intoxication have been studied *in vitro*, but little is known about how the toxins coordinate during infection to promote disease. Further, the etiology of *C. difficile*-associated diarrhea (CDAD) is attributed to cytoskeletal rearrangements but is not fully understood. Experiments using isogenic *C. difficile* toxin mutant strains in the mouse model of CDI revealed that both toxins were necessary to cause severe diarrhea. We hypothesized that each toxin dysregulates distinct junctional proteins and/or ion transporters that underlie CDAD. We used immunofluorescence of infected mouse tissues to determine that TcdB depletes NHE3 (Sodium hydrogen exchanger 3) in the distal colon, and that DRA (Down-regulated in adenoma) is depleted through a GT-independent mechanism. Preliminary RNA-sequencing analyses suggest that TcdA increases expression of *Claudin-2*, which is associated with increased paracellular leakage. Finally, we are using Üssing chambers to define toxin-dependent effects on ion transporter function and paracellular transport in tissue explants from infected mice. Results from these experiments will improve our understanding of CDAD, which may open new avenues for prevention and treatment of this debilitating symptom.

AN ANILINE-SUBSTITUTED BILE SALT ANALOG PROTECTS BOTH MICE AND HAMSTERS FROM MULTIPLE *CLOSTRIDIOIDES DIFFICILE* STRAINS

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Clostridioides difficile infection (CDI) is the major identifiable cause of antibiotic-associated diarrhea. The emergence of hypervirulent *C. difficile* strains has led to increases in both hospital- and community-acquired CDI. Furthermore, the rate of CDI relapse from hypervirulent strains can reach up to 25%. Thus, standard treatments are rendered less effective, making new methods of prevention and treatment more critical. Previously, the bile salt analog CamSA (cholic acid substituted with *m*-aminosulfonic acid) was shown to inhibit spore germination *in vitro* and protect mice and hamsters from *C. difficile* strain 630. Here, we show that CamSA was less active in preventing spore germination by other *C. difficile* ribotypes, including the hypervirulent strain R20291. The strain-specific *in vitro* germination activity of CamSA correlated with its ability to prevent CDI in mice. Additional bile salt analogs were screened for *in vitro* germination inhibition activity against strain R20291, and the most active compounds were tested against other strains. An aniline-substituted bile salt analog, CaPA (cholic acid substituted with phenylamine), was found to be a better antigerminant than CamSA against eight different *C. difficile* strains. In addition, CaPA was capable of reducing, delaying, or preventing murine CDI signs with all strains tested. CaPA-treated mice showed no obvious toxicity and showed minor effects on their gut microbiome. CaPA's efficacy was further confirmed by its ability to prevent CDI in hamsters infected with strain 630. These data suggest that *C. difficile* spores respond to germination inhibitors in a strain-dependent manner. However, careful screening can identify antigerminants with broad CDI prophylaxis activity.

AN ATKINS-TYPE DIET EXACERBATES *CLOSTRIDIOIDES DIFFICILE* INFECTION IN MICE

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Clostridioides [Clostridium] difficile infection (CDI) is responsible for the majority of antibiotic-associated diarrhea, a potentially lethal outcome. In recent years, overall incidences of CDI have risen to the point of surpassing methicillin-resistant *Staphylococcus aureus* (MRSA) as the most common hospital-associated infection. CDI affects over 500,000 people per year in the United States, resulting in over 29,000 deaths from CDI-related complications. This in turn contributes to a projected $4.8 billion cost burden to the U.S. healthcare system. CDI can result from the disruption of the resident gut microbiota. This can be brought on by the use of broad-spectrum antibiotics. Similarly, Western diets and popular weight-loss diets also drive large changes in the gut microbiome; however, there is conflicting literature on the effects of diets on CDI. In an antibiotic-induced mouse CDI model, we used spore from the hypervirulent strain *C. difficile* R20291 to assessed disease outcome and microbial community dynamics in mice fed various types of diets. Mice were fed either a high-fat/high-protein diet, a high-fat/low-protein diet, a high-carbohydrate diet, or a standard rodent diet. Both high-fat diets exacerbated CDI. A high-fat/high-protein, Atkins-like diet led to severe CDI signs and 100% mortality. A high-fat/low-protein, medium-chain triglyceride (MCT)-like diet induced highly variable CDI outcomes. In contrast to the high-fat diets, mice fed a high-carbohydrate diet were protected from CDI, despite high refined carbohydrate and low fiber content. Twenty-eight members of the *Lachnospiraceae* and *Ruminococcaceae* decreased in abundance due to diet and/or pre-spore-challenge antibiotic treatment; these organisms may compete with *C. difficile* for amino acids and protect healthy animals from CDI in the absence of antibiotics. Together, these data suggest that antibiotic treatment might lead to loss of *C. difficile* competitors and create a favorable environment for *C. difficile* proliferation and virulence that is intensified by high-fat/high-protein diets.

MICROBIAL COOPERATION ENHANCES *CLOSTRIDIOIDES DIFFICILE* PATHOGENESIS

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Clostridioides difficile is the most common nosocomial pathogen in the United States and an urgent global public health threat. Despite the well-established link between the gut microbiota and susceptibility to *C. difficile,* the impact of synergistic interactions between the gut microbiota and pathogens on the outcome of infection is largely unknown. The antibiotic-resistant enterococci are some of the most highly abundant members of the microbiota in the *C. difficile*-infected gut. We have found that Enterococcus abundance correlates with clinical metrics of severity in adults with *C. difficile* infection. Through a parallel process of nutrient restriction and cross-feeding, we hypothesize that enterococci shape the metabolic environment in the gut and reprogram *C. difficile* metabolism. Evidence of microbial cooperation between these two pathogenic organisms is observed in multiple murine models of infection, patient cohorts, and *in silico* metabolic modeling. Metabolomic analysis of *in vitro* co-culture systems and *in vivo* coinfection models reveals that nutrient exchange provides a reversible cue for *C. difficile* that facilitates increased virulence. These findings demonstrate that resident microbiota, such as Enterococcus, have a profound impact on *C. difficile* virulence and provide mechanistic insights into the cooperative roles of the microbiota on susceptibility to and severity of *C. difficile* infection.

DETERMINING THE EFFECTS OF YABG ALLELES ON THE CLEAVAGE OF *C. DIFFICILE* SLEC

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Clostridioides difficile forms metabolically dormant endospores, which allow for the dissemination from infected persons as well as resistance to harsh environments. Upon ingestion by the host, spores germinate into actively growing, toxin-producing vegetative cells. *C. difficile* spore germination is triggered in response to certain bile acids and amino acids [taurocholic acid (TA) and glycine]. In prior work, we identified CspA as the co-germinant receptor. CspA is encoded as a fusion with CspB – *cspBA*. Upon translation, YabG processes CspBA into CspB and CspA and, in addition to CspC, these proteins regulate the activation of the cortex-degrading enzyme SleC. YabG is a sporulation-specific protease that also processes preproSleC into proSleC. Interestingly, spores derived from *C. difficile* Δ*yabG* and *C. difficile* $yabG_{C207A}$ (catalytically inactive) strains germinate in response to TA alone and do not require co-germinants. Previously in an ethyl methanesulfonate (EMS) screen to identify mutants that did not germinate in response to TA, three mutations in *yabG* and two mutations in the *yabG* promoter sequence were identified. Recombinantly expressed and purified preproSleC incubated with *E. coli* lysate expressing wild type YabG resulted in the removal of the pre sequence from preproSleC. Further investigation is ongoing to determine the effects of the identified EMS mutant *yabG* alleles on germination and processing of recombinatly expressed preproSleC.

XENOSIDEROPHORES AS A SOURCE OF EXOGENOUS IRON IN *CLOSTRIDOIDES DIFFICILE*

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Clostridioides difficile is a Gram-positive, spore forming enteric pathogen, and the leading cause of antibiotic-associated nosocomial disease in the United States. *C. difficile* infection (CDI) causes gastrointestinal damage, inflammation, and perturbs nutrient dynamics between the host and commensal bacteria. To suppress pathogen and commensal outgrowth in the mammalian gut, the host restricts nutrient metals through a process termed nutritional immunity. Iron (Fe) is an essential nutrient metal for almost all life and is crucial for the structural integrity and enzymatic activity of important enzymes. Bacteria employ strategies to circumvent metal restriction in the mammalian intestine by synthesizing high affinity small molecule metal chelators, known as siderophores. Although siderophores are important in obtaining Fe during nutrient depletion from the mammalian host, *C. difficile* is incapable of manufacturing siderophores, but encodes for multiple putative siderophore transporters. We hypothesize that during CDI, *C. difficile* uses exogenously synthesized siderophores, also known as xenosiderophores, as an iron source to respond to periods of host mediated nutrient metal restriction. To combat siderophore-mediated Fe acquisition, the host produces Lipocalin-2 (LCN2), which binds iron loaded siderophores and transports them into host cells. Although LCN2 is highly expressed during intestinal inflammation its role in CDI is understudied. We anticipate that LCN2 restricts *C. difficile* growth by suppressing iron levels in the mammalian gut. In support of this, using an Fe restricted defined media, our preliminary data demonstrate that *C. difficile* can utilize multiple classes of siderophores as a sole source of Fe to rescue growth in Fe deplete conditions. This indicates that xenosiderophores may be vital for *C. difficile* persistence in the gut. We will determine the genes necessary for *C. difficile* to use xenosiderophores and combat LCN2 through the use of a transposon library, targeted mutagenesis, and the use of a $LCN2^{-/-}$ mouse model. In this study we will uncover the role of siderophores and LCN2 in *C. difficile* acquiring iron during CDI.

IDENTIFICATION AND CHARACTERIZATION OF A NON-ANTIBIOTIC TOXIN BIOSYNTHESIS INHIBITOR IN *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile is a spore-forming Gram-positive obligate anaerobe and a leading cause of healthcare-associated diarrhea. The pathogenesis of *C. difficile* results from toxins TcdA and TcdB that are responsible for epithelial damage and sporulation that promotes survival in the colon, which contributes to CDI recurrence. Front line treatment of CDI is based on treatment with antibiotics such as vancomycin and fidaxomicin. However, increasing resistance limits their efficacy. Recurrent CDI is treated with bezlotoxumab in combination with standard of care antibiotics or fecal microbiota transplant. However, there remains a need for alternate therapies to reduce the risk of recurrence.

To address the urgent need for alternative and adjunct treatments, we conducted a high-throughput screening (HTS) campaign to identify small molecules that are non-antibiotic but inhibit toxin biosynthesis in *C. difficile*. Preliminary screens identified a compound (compound 2) which inhibits *C. difficile* toxin production at an IC50 of 8 μM without substantial effect on growth. Compound 2 did not show any growth inhibitory properties against key gut bacterial species suggesting it has a narrow spectrum of activity. Compound 2 also inhibited *C. difficile* sporulation at 8 μM and above. To understand the mode of action of compound 2, we analyzed transcriptome changes by RNAseq analysis. Compound 2 was found to interfere with carbon metabolism, evident by downregulation of glycolysis, upregulation of galactitol and fructose transporters. We will present an in-depth mechanism of the anti-virulence and mode of action of compound 2 in *C. difficile*.

A GUT MICROBIOTA SHAPED BY INFLAMMATION IS PERMISSIVE FOR COLONIZATION BY *CLOSTRIDIOIDES DIFFICILE*

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Introduction: Perturbations of the gut microbiota including those results from antibiotics, leads to susceptibility to *Clostridioides difficil*e. The gut microbiota are also implicated in the pathogenesis of inflammatory bowel disease (IBD). Patients with IBD are at increased risk for developing *C. difficile* infection, even in the absence of antibiotics. We recently have developed a model of *C. difficile* susceptibility in IL-10-deficient mice that develop colitis when colonized with *Helicobacter hepaticus.* We found that mice with colitis were susceptible to *C. difficile* colonization in the absence of antibiotic treatment.

Methods: To test the hypothesis that treating inflammation restores resistance to *C. difficile* colonization in the setting of IBD, colitic mice were treated with a monoclonal antibody (mAb) targeting the p40 subunit shared by the pro-inflammatory cytokines, IL-12 and IL-23. The susceptibility to *C. difficile* colonization of mice treated with the anti-p40 mAb were compared to animals treated with an isotype control antibody.

Results: Gut microbiota structure was altered in mice with IBD. anti-p40 treatment reduced inflammation, and this was associated with a shift in community composition back toward baseline. Control mice with active IBD were more susceptible to *C. difficile* colonization relative to anti-p40-treated mice. Notably, microbiota composition correlated with *C. difficile* susceptibility in mice IBD, regardless of treatment status. We confirmed these findings by conducting fecal transplants in germfree C57BL/6 mice. We found that the susceptibility of fecal microbiota transplant recipients to *C. difficile* colonization matched that of their donors. Machine learning models identified protective taxa that were altered by inflammation that overlapped with taxa identified in antibiotic treated animals that were susceptible to *C. difficile*.

Conclusions: These results suggest that inflammation sculpts the microbiota to create a permissive landscape for *C. difficile* colonization in the setting of IBD. Improved understanding of how inflammation and the gut microbiota influence colonization resistance will aid in the development of strategies to prevent or treat *C. difficile* infection in the IBD patient population.

July 28-31

Sunday, July 31 — *Clostridium* ssp

1315-1415 Poster Session II: *Clostridium* ssp

Posters will be presented in Poster Session II
Sunday, July 31 1315-1415.

DIVERSITY AND PREVALENCE OF *CLOSTRIDIUM INNOCUUM* IN THE HUMAN GUT

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Of the thousands of bacterial species inhabiting human gastrointestinal tract, many belong to the class Clostridia, a polyphyletic group of Gram-positive, spore-forming anaerobes in the Firmicutes phylum. Recently, Clostridia were divided into two separate classes, Clostridia and Erysipelotrichia, based on phenotypic and 16S rRNA gene-based differences. While Clostridia include many well-known pathogenic bacteria, Erysipelotrichia remain relatively uncharacterized, particularly regarding their role as a pathogen vs. commensal. The Erysipelotrichial species *Clostridium innocuum*, even though it is widely recognized as a commensal, has been identified as a potential cause of diarrhea in patients with prior *Clostridioides difficile* infection and has been associated with other inflammatory gastrointestinal conditions, such as Crohn's disease. To further understand the ecological and potential clinical role of *C. innocuum*, we conducted a genomic comparison across *C. innocuum* isolates and publicly available genomes. Based on colony morphology, we isolated multiple *C. innocuum* cultivars (n=38) from the feces of healthy human volunteers (n=7). Comparison of the 16S rRNA gene of our isolates against publicly available microbiota datasets in healthy individuals suggests a high prevalence of *C. innocuum* across the human population (> 85%). Analysis of single nucleotide polymorphisms (SNP) across core genes and average nucleotide identify (ANI) revealed the presence of 4 clades among our strains and additional available genomes (n=113 total). Investigation of carbohydrate and protein utilization pathways, including comparison against the carbohydrate-activating-enzyme (CAZyme) database, demonstrated inter- and intra-clade differences. Although previous studies suggest a potential pathogenic role for *C. innocuum,* comparison of our isolates against multiple databases of known virulence factors did not identify putative *C. innocuum* toxins. Collectively, these data indicate genetic variance within the *C. innocuum* species that may help clarify its role in human disease and health.

INTRA-SPECIES DIVERSITY OF *CLOSTRIDIUM PERFRINGENS*: A DIVERSE GENETIC REPERTOIRE REVEALS AN EMERGING PATHOGENIC POTENTIAL

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The purpose of this study was to determine the intra-species diversity and phylogenetic relationships of *C. perfringens,* as well as to identify key molecular markers associated with pathogenicity, virulence, and antibiotic markers resistance (AMR) from Whole Genome Analysis (WGA).

In this study, 372 *C. perfringens* genomes from multiple locations and sources were evaluated. In silico MLST was used for typing and the resulting sequence types (STs) were assigned to clonal complexes (CC). A pangenome analysis was conducted and then a core genome-based phylogenetic tree was created to define phylogroups. Additionally, toxinotypes, other key virulence factors (VF), and AMR were identified using ABRicate.

Most of the *C. perfringens* genomes analyzed in this analysis were derived from food (n=85) and bird (n=85). A total of 195 STs, some of them shared between sources such as food and human, horses and dogs, as well as environment and birds, were grouped in 25 CCs and distributed along five phylogroups. The 53% of the genomes were allocated to toxinotype A, followed by F (32%), and G (7%). The VF most frequently found was alpha-toxin (100%), followed by sialidase nanH (99%), and alpha-clostripain (99%), while tetA (39.5%) and tetB (36.2%), which mediate tetracycline resistance, were the most common AMR detected.

This analysis showed a better view about the possible spread of this pathogen among hosts, either by direct contact or by contaminated food. Furthermore, our results confirm a great diversity of *C. perfringens* with many virulence factors that vary among different phylogroups and AMR, especially to tetracyclines, aminoglycosides, and macrolides, which highlight its importance at the public health level as an emerging pathogen.

NANJ IS THE MAJOR SIALIDASE FOR SOME *CLOSTRIDIUM PERFRINGENS* TYPE F FOOD POISONING STRAINS

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Clostridium perfringens type F food poisoning (FP) strains produce *C. perfringens* enterotoxin (CPE) when they sporulate in the intestines. *C. perfringens* can also produce up to three different sialidases, named NanH, NanI, and NanJ. Our previous study (1) surveyed FP strains, which showed that most of these strains carry only the *nanH* sialidase gene, although others carry both the *nanJ* and *nanH* sialidase genes. In another study, we showed that NanH is produced only by sporulating cultures of FP strains carrying only the *nanH* gene and is not necessary for either the growth or sporulation of those FP strains. NanH was shown to accumulate in the sporulating mother cell of those strains until it is released coincidently with CPE. Furthermore, NanH was shown to increase CPE binding and cytotoxicity for Caco-2 cells.

In this study, we studied the FP strains that carry both the *nanJ* and *nanH* sialidase genes. The results showed that, in both vegetative and sporulation culture, NanJ is the major sialidase produced by these strains. We used sialidase mutants to show that, in vegetative cultures, NanH production represses NanJ expression, but NanH production positively regulates NanJ expression in the early stage of sporulation. It was also determined that NanJ contributes to FP strains growth or survive in both vegetative and sporulating cultures. Overexpression of NanJ was shown to inhibit sporulation of these FP strains. This ongoing study is now studying how NanJ contributes to CPE binding and cytotoxicity for Caco-2 cells.

Reference:

Li J, McClane BA. NanH is produced by sporulating cultures of *Clostridium perfringens* type F food poisoning strains and enhances the cytotoxicity of *C. perfringens* enterotoxin. mSphere. 2021 28;6(2): e00176-21. doi: 10.1128/mSphere.00176-21. PMID: 33910991.

THE OCCURRENCE OF CLOSTRIDIAL SPORES IN THE UROLOGY HOSPITAL ENVIRONMENT (SOUTHERN POLAND) DURING SARS-COV-2 PANDEMIC

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The aim of this study was to detect the presence of clostridial spores in 86-bed urology hospital environment (XI, 2020), with the use of a special C diff Banana Broth ™ (Hardy Diagnostics, Santa Maria, CA USA) and to evaluate antibiotic susceptibility and toxin profile of isolates.

The study was performed few weeks after closing the hospital for 2 weeks for H_2O_2 fogging, because of SARS-CoV-2 infection cases among medical staff. 58 swabs from hospital environment were collected to the C diff Banana Broth™, incubated at 370 C for 14 days, checking every 24 h. After identification and the MIC values determination by E-tests, mPCR for toxin genes of *C. difficile* and *C. perfringens* isolates were performed.

16 strains of *Clostridium* spp. (~28%) were cultured: 11 strains of *C. perfringens*, 2 – *C. baratii* and one strains each of *C. paraputrificum, C. difficile* and *C. clostridioforme.* Among *C. perfringens* strains 9 possessed the *cpa* gene, 7the *cpb2* gene and 2*cpiA* gene and 1 *cpb* gene. *C. difficile* strain possessed *tcdA*, *tcdB and tcdA/tcdB* genes. All isolates were sensitive to metronidazole, vancomycin, moxifloxacin, penicillin and rifampicin. 6/11 *C. perfringens* strains were resistant to erythromycin, 2/11 – to clindamycin. *C difficile* was resistant to imipenem and piperacillin/tazobactam. One of 2 *C. baratii* was resistant to erythromycin and piperacillin/tazobactam and *C. paraputrificum* - to piperacillin/tazobactam.

The presence of *Clostridium* spp. spores in a hospital environment was confirmed in 27.6%, (69% – *C. perfringens*), regardless of the H_2O_2 fogging. The high frequency of *C. perfringens* spores, with a much lower sporulation capacity compared to *C. difficile*, indicates favorable conditions for sporulation. Regardless of the lack of *Clostridium* spp. (other than *C. difficile* and 1 case of *C. perfringens*) in the materials collected from patients, antibiotic-resistant and toxigenic strains of *C. perfringens* were cultured from the samples from hospital environment, indicating the need to develop the necessary sanitary and epidemiological procedures in this hospital. For epidemiological surveillance appropriate media such as C diff Banana Broth™ for detection of clostridial spores should be used, independently of studied hospital/ward type.

IMMUNOMODULATORY CLOSTRIDIA ISOLATED FROM HELMINTH-COLONIZED HUMANS PROMOTE HATCHING OF *TRICHURIS MURIS*

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Soil transmitted intestinal worms known as helminths colonize over 1.5 billion people worldwide. Although helminth colonization in both humans and mice has been associated with altered composition of the gut microbiota, such as increases in Clostridia, individual species have not been isolated and characterized. Clostridia are a class of spore-forming obligate anaerobes that have previously been shown to have anti-inflammatory properties through their ability to induce regulatory T cells (Tregs). To better characterize these bacteria in the context of helminth infection, we isolated and sequenced the genome of 13 Clostridia from the Orang Asli, an indigenous population in Malaysia with high prevalence of helminth infections. Metagenomic analysis of 650 fecal samples from urban and rural Malaysians revealed higher prevalence and abundance of these isolates compared to individuals in the United States, with *Peptostreptococcaceae* family members displaying a specific association with helminth colonization. Monocolonization of Treg-deficient germ-free mice with these isolates also resulted in an increase of Rorγ+ Helios- Tregs, which are induced by microbial antigens in the colon. Remarkably, *Peptostreptococcaceae* isolated from the Orang Asli displayed superior capacity to induce hatching of eggs from the murine helminth *Trichuris muris*. These findings support a model in which helminths select for gut colonization of immunomodulatory microbes that in turn support their life cycle by promoting egg hatching.

INVESTIGATION OF INTERACTIONS BETWEEN ENTEROPATHOGENIC CLOSTRIDIA AND HUMAN GASTROINTESTINAL BACTERIA

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Antibiotic treatments against bacterial infections of the gut often not only target the pathogens but also affect commensal bacteria. This can induce dysbiosis and diseases. The Gram-positive, spore-forming bacteria of the genus *Clostridium* are ubiquitously found in soil, intestinal tract of animals, water, and other biotopes. Among them are rapidly growing, anaerobic human pathogens, associated with intestinal diseases and gas gangrene in contaminated wounds. Drug-efficacy against specific microbes can differ in microbial communities and axenic cultures. This was also observed for clostridia treated with different antibiotics. Properties that emerge from interactions within the community can be of protective (cross-protection) or sensitizing (cross-sensitization) nature, but the underlying mechanisms are often unknown. This project aims to unravel interactions of clostridial strains of interest with 17 prevalent and abundant, and therefore representative human gut microbes. To characterize interactions between the different species, we genetically modified strains of interest to express the fluorescence-activating and absorption-shifting (FAST) tag. Those strains are fluorescent in the presence of a FAST-ligand and, are thus investigated for simplified tracking in communities. In parallel, the growth of the same strains is monitored in co-cultures and in communities by qPCR with species-specific primers. It is sought to identify beneficial and adverse effects on the growth rate of the strains of interest in co-cultivation with other human gut microbes. The molecular mechanisms underlying the observed interactions will be investigated through a combination of genetic, and different omics approaches. The ultimate goal of the project is to understand the different outcomes of drug treatment in axenic culture or communities and to find new treatment approaches with probiotic strains, communities, or specifically engineered strains.

TARGETED ISOLATION OF CLOSTRIDIA FROM HUMAN FECES

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Sequencing technology has provided insight into the bacteria inhabiting the gut, yet cultivation efforts fall short for characterization of this diverse community. The aim of this study was to develop a standardized method for isolating anaerobic bacteria from feces that targets a particularly undercharacterized group of bacteria, Clostridia. We tested the ability of five different media to select for different species of Clostridia: Bovine Heart Infusion (BHI), BHI supplemented with clarified rumen fluid, Reinforced Clostridial Medium (RCM), Yeast Extract, Casitone and Fatty Acid medium (YCFA) with taurocholate, and Taurocholate Cycloserine-Cefoxitin-Fructose (TCCFA). Fecal slurries were inoculated on both solid and liquid media of each media and incubated anaerobically for 24 hours. Broth inoculations were diluted and plated on corresponding solid media. Single isolated colonies were picked based on unique morphology and restreaked for isolation until visually pure, then repicked into broth for storage and downstream applications. The full-length 16S rRNA gene was PCR-amplified for initial taxonomic identification by Sanger sequencing. Using five different fecal samples collected from healthy individuals, we isolated over 400 morphologically distinct cultivars, 64% of which were classified as Clostridia or Erysipelotrichia, with 47% of isolates identified as Lachnospiraceae species. Lachnospiraceae genera Anaerostipes (17%), Dorea (12%), Blautia (17%) and Roseburia (1%) were represented across and within individual fecal samples. Of the isolated Lachnospiraceae cultivars, YCFA yielded the most (36%), followed by BHI (35%), and BHI + rumen fluid (35%). BHI, however, yielded the most diverse species overall, with 93 unique cultivars. Collectively, these results support development of standardized methods for targeted isolation of fecal bacteria. We aim to expand these results towards other hosts to maximize Clostridial representatives of the gut.

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