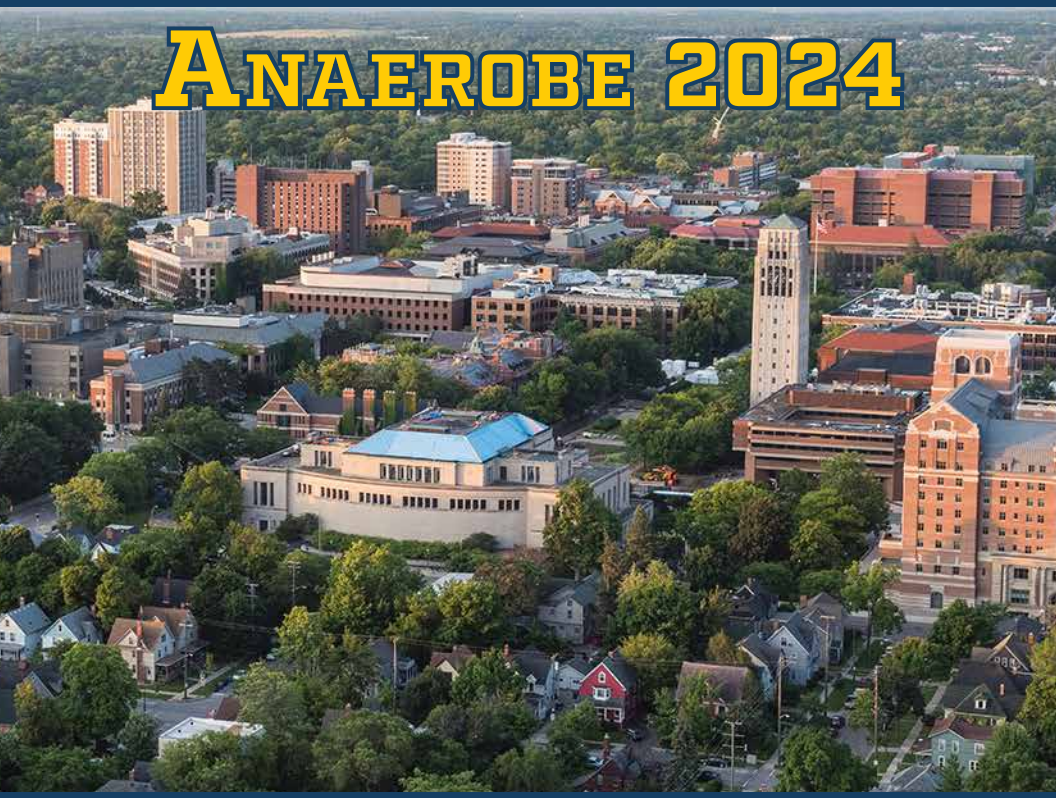
Program and Abstract Book

ANAEROBE 2024



The 17th Biennial Congress of the Anaerobe Society of the Americas

University of Michigan,
Ann Arbor, MI USA
July 8-11, 2024

Program and Abstract Book

ANAEROBE 2024

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Contents

ANAEROBE 2024
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The 17th Biennial Congress of the Anaerobe Society of the Americas

Dear Colleagues:
Welcome to Ann Arbor and ***ANAEROBE 2024***, the 17th biennial Congress of the Anaerobe Society of the Americas (ASA). This forum brings together clinicians and scientists from around the world to engage in presentations, dialogue, and interaction related to the clinical and microbiological aspects of anaerobic bacteriology.

The Congress will explore the role of anaerobes in both health and disease, and address traditional and emerging technologies for the identification, diagnosis, and scientific study of these important microbes. ***ANAEROBE 2024*** again illustrates the international interest in the field of anaerobic bacteriology: over 140 abstracts were submitted for presentation, representing the work of more than 650 scientists from over 20 countries.

The Keynote Address will be given by noted physician and researcher **R. Balfour Sartor, MD** of the University of North Carolina at Chapel Hill. Dr. Sartor has made critical contributions to our understanding of the role of the indigenous gut microbiota in health and disease. He will discuss how there has been a therapeutic transition from targeting gastrointestinal pathogens with antibiotics to promoting colonization resistance and restoring healthy microbial metabolic function to promote homeostasis.

The *Lifetime Achievement Award* will be presented to **Ellie J.C. Goldstein, MD** of the RM Alden Research Laboratory and the University of California, Los Angeles. Dr. Goldstein is the co-founder of the Anaerobe Society of the Americas (with Dr. Sydney Finegold), and his work studying anaerobic bacteria and their infections is documented in over 480 publications.

I am grateful for the work of the members of the Organizing Committee and the Session Chairs for formulating what promises to be a dynamic and exciting program. The ASA and I also would like to thank those from industry—both patrons and exhibitors—for the financial support that makes this Congress possible.

I especially wish to thank Dr. Ronald and Pamela Goldman, who have guided the society and organized these Anaerobe Congresses over the past 25 years. The growth and success of ASA and this biennial congress has been dependent on their continued efforts over the years. In addition, Karrie Black of the University of Michigan provided valuable assistance in preparation for this meeting in Ann Arbor.

The Anaerobe Congresses have long been very successful in not only serving as a forum for stimulating discussions on clinical and research topics relating to anaerobic bacteriology, but in fostering networking and inter-organizational collaborations to further the field. Our hope is that ***ANAEROBE 2024*** will continue this tradition.

Vincent Young, MD, PhD
President, Anaerobe Society of the Americas

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 17th biennial Anaerobe Society Congess.
Past Anaerobe Society sponsored programs were:

ANAEROBE 2022—Seattle, WA USA
ANAEROBE 2020—Virtual
ANAEROBE 2018—Las Vegas, NV USA
ANAEROBE 2016—Nashville, TN USA
ANAEROBE 2014—Chicago, IL USA
ANAEROBE 2012—San Francisco, CA USA
ANAEROBE 2010—Philadelphia, PA USA
ANAEROBE 2008—Long Beach, CA USA
ANAEROBE 2006—Boise, ID USA
ANAEROBE 2004—Annapolis, MD USA
ANAEROBE OLYMPIAD 2002—Park City, UT USA
2001: AN ANAEROBE ODYSSEY—Los Angeles, CA USA
ANAEROBE 2000—Manchester, England
ANAEROBE 1998—Buenas Aires, Argentina
ANAEROBE 1996—Chicago, IL USA
ANAEROBE 1994—Los Angeles, CA USA
ANAEROBE 1992—Los Angeles, CA USA

For Additional Information

About the Anaerobe Society or *Anaerobe 2024*

Web Site: *www.anaerobe.org*

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Phone +310-216-9265

Patrons & Exhibitors

Anaerobe Society of the Americas gratefully acknowledges the following organizations for their generous support of the 17th biennial Congress.

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Support for this activity was received in the form of Educational Grants from:

- European Society of Clinical Microbiology and Infectious Diseases
  www.escmid.org

Patrons:

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  www.sciencedirect.com/journal/anaerobe
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- Beijing Longfujia Life Sciences Ltd.
  www.longfubiotech.com/eindex.asp
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  www.ferring.com
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  www.innovivaspecialtytherapeutics.com
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  www.800ezmicro.com
- Nestle Health Sciences
  www.nestlehealthsciences.com
- Plas Labs
  www.plas-labs.com
- Vedanta Biosciences
  www.vedantabio.com

Keynote Speaker

R. Balfour Sartor, MD

R. Balfour Sartor, is the Midget Distinguished Professor of Medicine, Microbiology, and Immunology in the Division of Gastroenterology and Hepatology at the University of North Carolina (UNC) at Chapel Hill, NC, USA.

Dr. Sartor received his MD from Baylor College of Medicine, where he completed his Internal Medicine residency, prior to pursuing a combined clinical and research Gastroenterology fellowship at UNC. He is Co-Director for the NIDDK-funded Center for Gastrointestinal Biology & Disease, Director of the NIH-funded National Gnotobiotic Rodent Resource Center, and was the founding Director of the UNC Multidisciplinary IBD Center.

His clinical interests are in treating difficult to manage IBD patients, with ongoing translational research to identify microbial and genomic biomarkers that predict risk of post-operative recurrence of Crohn's disease and microbial factors associated with recurrent pouchitis. His research focuses on developing and applying rodent models of chronic, immune-mediated intestinal inflammation relevant to IBD and performing clinically relevant translational studies involving IBD patients.

Dr. Sartor investigates genetically-determined immune responses to luminal resident bacterial subsets using gnotobiotic mice and patient-derived samples, and studies the influence of environmental factors, including diet, on intestinal microbiota composition and function. Dr. Sartor has published over 400 articles, editorials, chapters and has edited 5 books. Dr. Sartor has trained over 60 postdoctoral fellows and graduate students. In addition, he has led multiple large multidisciplinary consortium basic studies that have extensively impacted the understanding of IBD pathogens. In 2020, he received the American Gastroenterological Association's Distinguished Achievement Award in Basic Science.

Lifetime Achievement Award

Ellie J.C. Goldstein, MD FSHEA, FIDSA

Ellie Goldstein is in private practice in Santa Monica, CA, USA. He was Director of RM Alden Research Laboratory (Diane M. Citron, Assoc. Director), and Clinical Professor of Medicine, UCLA School of Medicine (Former).

His clinical and research interests include anaerobic bacteria and their infections, such as *C. difficile* infections (epidemiology, prevention and therapy); human and animal bite wounds (bacteriology, clinical presentations, complications and therapy); bacteriology and therapy of intra-abdominal infections; diabetic foot infections; *in vitro* susceptibility of fastidious and anaerobic bacteria to new antimicrobial agents; and pharmacokinetics and pharmacodynamics of new agents. He has over 480 publications including in the *New England Journal of Medicine, Science, JAMA, PLoS, Lancet ID* and *Clinical Infectious Diseases*.

Dr. Goldstein founded, and served as President, of the Infectious Diseases Association of California (IDAC) (1985-1991) and, with Sydney Finegold, co-founded the Anaerobe Society of the Americas (1992).

Dr. Goldstein Chairs ASP and Infection Prevention for Kindred LA Hospital and Providence St Johns' Health Center. He served on the IDSA Council and received the IDSA *Watanakunakorn Clinician Award* (1995).

He was the Clinical Practice Section Editor for *Clinical Infectious Diseases* (2000-2022) and was Chair of the Publications Committee of *Anaerobe*. In the past he has served as an Associate Editor for *Clinical Infectious Diseases* (1999-2000) and the *Journal of Medical Microbiology and Infectious Diseases in Clinical Practice*.

In Memoriam

Joseph S. Solomkin, MD, FACS, FIDSA

Joseph S. Solomkin, MD, FACS, FIDSA was among the leading authorities on surgical intra-abdominal infections.

He completed his undergraduate studies at Harvard University and received his MD from Albert Einstein College of Medicine in New York. His surgical training was done at the University of Minnesota School of Medicine in Minneapolis, MN, before serving in the U.S, Army Medical Corps, including deployment to Vietnam during the Vietnam War.

In 1981, he joined the Department of Surgery at the University of Cincinnati College of Medicine in Cincinnati, OH. He arrived as a general surgeon, specializing in burns and trauma, but over his more than 25 years as a professor, he developed an interest in surgical infections and became the Director of the Division of Surgical Infections. He published extensively on the mechanisms and management of surgical infection, earning an H-index of 61, by co-authoring 214 publications receiving 15,419 citations.

Dr. Solomkin was involved in the evaluation of a range of antimicrobial agents and in the development of guidelines for the selection of anti-infective agents for the treatment of surgical infections. He actively participated in formulating the WHO Guidelines for the Prevention of Surgical Site Infections and coordinated the IDSA guidelines for the management of intra-abdominal infections.

He was an active member of a number of medical societies, including the American College of Surgeons, the Society for Critical Care Medicine, the American Society for Microbiology, the Infectious Diseases Society of America, the Society for Leukocyte Biology, the Surgical Infection Society, and the Anaerobe Society of the Americas. He was the keynote speaker at ***ANAEROE 2016***, on the topic of *New Agents for Complicated Intra-Abdominal Infections*.

He also founded the World Surgical Infection Society/Oasis Global Inc., an international organization whose mission is to support WHO guidelines in bringing affordable and effective infection control practices to low and middle-income countries, such as reducing hospital-acquired surgical maternal and neonatal infections following caesarean section in eastern and southern Africa countries. This organization grew to offer services in 13 countries, worldwide.

Dr. Solomkin passed away on November 4, 2023, at the age of 79.

ANAEROBE 2024—The 17th biennial Congress of the Anaerobe Society of the America+s—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).

No Continuing Education Units will be issued for this Congress. For those requiring documentation of attendance, delegates may request Certificates of Attendance, free of charge, on their Evaluation Form, and certificates will be emailed after Congress evaluations are processed. Certificates will only be issued to those returning Evaluation Forms, by the end of the Congress.

Disclosures

Disclosures of relevant financial relationships by all session participants are provided on pages xiii-xiv.

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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Katherine Winner
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Vincent B. Young, MD, PhD
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Disclosurer Information

This Congress has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME). The Anaerobe Society of the Americas (ASA) has attempted to ensure balance, independence, objectivity, and scientific rigor in this continuing medical education activity. All individuals in a position to control the educational content of this activity, as well as all oral presenters, have disclosed to ASA any financial interests or other relationships they have had in the past 12 months with commercial interests whose product(s) will be referred to in presentations, may be providing educational grants, or 'in-kind' support of this activity.

Although the existence of a commercial interest relationship in itself does not imply bias or decrease the value of presentations, this information is provided to the audience to allow them to make their own judgments. It remains for the audience to determine whether the speaker's interest or relationships may influence the presentation with regard to exposition or conclusion.

The ACCME Standards for Commercial Support require that presentations be free of commercial bias and any information regarding commercial products/services be based on scientific methods, generally accepted by the medical community. If a presentation has discussion of unlabeled/investigational use of a commercial product, that information must be disclosed to the participants of the activity.

The disclosure information received from each individual is presented on the following pages. All disclosure information has been reviewed for conflict of interest by the ASA Program Committee.

Participant Disclosurers

The following presenters do not have financial relationships with commercial interests; no relationships between commercial interests and first degree relatives exist, and do not intend to discuss an unapproved/investigative use of commercial product/device:

Michelle Adamczyk
Caetano Antunes, PhD
Leah Beauchamp, PhD
Kevin Bollinger
Charles Booth, Jr., PhD
Rishi Chanderraj, MD
Deiziane Viana da Silva Costa
Michael Dieterle, MD, PhD
Qiwen Dong, PhD
Adrienne Edwards, PhD
J. Christopher Fenno, PhD
Eliane de Oliveira Ferreira, PhD
Yousi Fu, PhD
Ronald Goldman, PhD
Yiping Han, PhD
Jessica Hastie, PhD
Yolanda Yue Huang, PhD
Sarah Kuehne, PhD
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Aimee Shen, PhD
Evan Snitkin, PhD
Francisco Uzal, DVM, PhD
Harold Weisenfeld, MD
Katherine Winner
Suemin E. Yang

The following presenters have information to disclose as follows:

Laura M. Cox, PhD	Anaerobe Systems (E)
David Fredricks, MD	BD (R), MetaboliteDx (E)
Kevin Garey, PharmD	Acurx (C, G), Paratek (C, G) *Speaking on the clinical trial development of ibezapolstat for the treatment of CDI*
Holly Ganz, PhD	AnimalBiome (E, O)
Ellie J.C. Goldstein, MD	Acrux (C), Merck (C, S), Melinta (C, S), Shionogi (C, S)
Jinhee Jo, PharmD	None *Speaking on omadacycline in the context of CDI*
Stuart Johnson, MD	Acurx (C), BioK+ (C), Ferring (C)
William Johnston, PhD	None *Speaking on new therapeutic uses for previously developed compounds for bacterial vaginosis*

Ryan Kean, PhD	Ferring (G)
Glynis Kolling, PhD	Cerillo (C)
Rajat Madan, MD, PhD	Medpace (C)
Nirja Mehta, MD	None *Speaking on some microbiome therapuetics which have completed Phase 2 testing*
Daniel Paredes-Sabja, PhD	LivProcess (C)
Krishna Rao, MD	Merck (G), Rebiotix (C), Seres (C), Summit (C)
R. Balfour Sartor, MD	Biomica (C, G), Gusto Global (G), ImmunyX (G)
Cynthia Sears, MD	Janssen (G), Bristol Myers Squibb (G)
Sujatha Srinivasan, PhD	MetaboliteDx
Julia Swavola, PhD	Cerrillo (E)
Casey M. Theriot, PhD	Ancilia Biosciences, Inc (C, G) Vedanta Biosciences (C)
Cirle Warren, MD	Ferring (G)
Vincent B. Young, MD	Aimmune (C), Debiopharm (C), Vedanta Biosciences (C, G)

A=Advisory Board, C=Consultant, E=Employment, G=Grant, O=Ownership/Stock, P=Patent, R=Royalty, S=Speaker

Monday, July 8

0800

WORKSHOPS & CONGRESS REGISTRATION OPENS

0900-1700

ANAEROBIC IDENTIFICATION & SUSCEPTABLITY WORKSHOP

Laura M. Cox, PhD

0900-1700

EXAMINING ANAEROBES IN THE MICROBIOME: METAGENOMIC AND CULTURE APPROACHES

Anna Seekatz, PhD

Casey M. Theriot, PhD

Evan S. Snitkin, PhD

1800-1900

SESSION I: CONGRESS KEYNOTE

Moderator: *Vincent B. Young, MD, PhD*

SI-1 Evolution of Thinking about the Role of Anaerobic Microbes in Gastrointestinal Health and Disease

R. Balfour Sartor, MD

1900

KEYNOTE RECEPTION & BUFFET DINNER

Michigan League on the University of Michigan Campus

ANAEROBE 2024
July 8-11, 2024

Tuesday, July 9

0700-0820 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS

0820

WELCOME REMARKS
Vincent B. Young, MD, PhD, ASA President

0830-0930

SESSION II: ANAEROBES: DISEASE ASSOCIATIONS
Moderator: *Vincent B. Young, MD, PhD*

SII-1 Roles of Three *Fusobacterium nucleatum* Subspecies in Colorectal Cancer
Sarah Kuehne, PhD†

SII-2 *Clostridioides difficile* Infection Triggers Late Neuroinflammatory Response in the Brain
Suemin E. Yang

SII-3 Investigating the Role of the Gut Microbiota in the 3KL Model of Parkinson's Diseases Using Selective Antibiotic Depletion
Leah Beauchamp, PhD

0930-1030

SESSION III: ANAEROBIC INFECTIONS
Moderator: *Yiping Han, PhD*

SIII-1 Finding *Fusobacterium necrophorum:* A Journey of Patient and Pathogen Discovery
Michael Perry†

SIII-2 *Fusobacterium nucleatum* is Enriched in Invasive Colorectal Cancer Biofilms
Jessica Queen, MD, PhD

SIII-3 Strategic Drug Repurposing for Biofilm Associated Bacterial Vaginosis
William Johnston, PhD

1030-1045 BREAK / INDUSTRY EXHIBITS

1045-1145

SESSION IV: ANAEROBIC CLINICAL INFECTIONS
Moderator: *Kevin Garey, PharmD*

SIV-1 ***Lifetime Achievement Lecture***
Decreasing Needless Self-Flagellation: Overuse of Metronidazole in Aspiration Pneumonia
Ellie J.C. Goldstein, MD

SIV-2 Serum and Stool Inflammatory Mediators are Viable Indicators of Risk for Developing Recurrent *Clostridioides difficile* Infection
Michael Dieterle, MD, PhD

SIV-3 Comparing Mortality of Sepsis Patients Administered Piperacillin-Tazobactam or Cefepime Using Instrumental Variable Analysis
Rishi Chanderraj, MD

1145-1315 LUNCH / INDUSTRY EXHIBITS

1200-1300

SATELLITE SESSION
How to Publish Your First Manuscript (in *Anaerobe*)
Emma Allen-Vercoe, PhD

†Sponsored by European Society of Clinical Microbiology & Infectious Diseases (ESCMID)

The 17th Biennial Congress of the Anaerobe Society of the Americas

Tuesday, July 9

SESSION V: ANAEROBES IN HEALTH & DISEASE
Moderator: *Laura Cox, PhD*

1315-1415

SV-1 Deleted in Malignant Brain Tumors 1 Glycoprotein is Lost in Colonic Dysplasia
Nicholas Markham, MD, PhD

SV-2 *In silico* Design of Bacterial Consortia Mitigates *Clostridioides difficile* Pathogenesis in a Murine Model
Glynis Kolling, PhD

SV-3 NOMMS: The Nasal and Oral Microbiome in Multiple Sclerosis
Shuqi Li, PhD

1415-1430 BREAK / INDUSTRY EXHIBITS

SESSION VI: *CLOSTRIDIOIDES DIFFICILE* I: CLINICAL/EPIDEMIOLOGY UPDATES
Moderator: *Stuart Johnson, MD*

1430-1530

SVI-1 Evolution of the Treatment of CDI: From Metronidazole to FDA-Approved FMT
Krishna Rao, MD

SVI-2 Clinical Efficacy of Ibezapolstat in CDI: Results from Phase 2 Trials
Kevin Garey, PharmD

SVI-3 Molecular Epidemiology of *Clostridioides difficile* in the United States, 2020
Michelle Adamczyk

1530-1730 POSTER SESSION I / INDUSTRY EXHIBITS

1730-1830 ASA BUSINESS MEETING

Nichols Arboretum
1600 Washington Hts.

Frankel Detroit Observatory
1398 E. Ann St.

Wednesday, July 10

0700-0800 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS

0800-0900

SESSION VII: INTERSPECIES & MICROBE/MICROBE INTERACTIONS

Moderator: *Casey M. Theriot, PhD*

SVII-1 Bioactive Compounds Produced by the Gut Commensal *Enterocloster citroniae* Modulate Enteric Pathogen Virulence
Caetano Antunes, PhD

SVII-2 Protection Against CDI Colitis in a Mouse Model by a Clinical Avriulent *Clostridioides difficile* Isolate through Amino Acids Competition
Qiwen Dong, PhD

SVII-3 *Fusobacterium nucleatum* and *Clostridioides difficile* Co-Colonization Increases Inflammation of the Gut Epithelium
Charles Booth, Jr., PhD

0900-1000

SESSION VIII: OTHER ANAEROBES: PHYSIOLOGY & CLINICAL ASSOCIATIONS

Moderator: *Purnima Kumar, DDS, PhD*

SVIII-1 Analyzing the *Treponema denticola* Protease Complex (Dentilisin) in Clinical Isolates
J. Christopher Fenno, PhD

SVIII-2 *Bacteroides thetaiotaomicron* Prevents Death from *Clostridioides difficile* Infection in Aged Host: Potential Role of Interferon Signaling
Cirle Warren, MD

SVIII-3 Antimicrobial Susceptibility of Gram Negative Anaerobic Isolates in a High Complexity Pediatric Hospital from Buenos Aires, Argentina
Silivina Otera

1000-1015 BREAK / INDUSTRY EXHIBITS

1015-1115

SESSION IX: ONE HEALTH: ANAEROBES IN ANIMALS & MAN

Moderator: *Francisco Uzal, DVM, PhD*

SIX-1 The Epidemiology of *Clostridioides difficile* in a One-Health Context
Eliane de Oliveira Ferreira, PhD

SIX-1 Effects of Commonly Used Medications on the Growth of Commensal Gut Anaerobes and Pathobionts Associated with Domestic Cats and Dogs
Holly Ganz, PhD

SIX-1 The Microbiome & Resistome of Golden Jackals in Israel
Katherine Winner

The Huron River offers kayaking, canoeing, fishing, hiking, and biking.

Wednesday, July 10

SESSION X: ANAEROBES IN REPRODUCTIVE HEALTH

Moderator: *David Fredricks, MD*

1115-1215

SX-1 The Importance of Anaerobic Antimicrobial Therapy for Acute Pelvic Inflammatory Disease (PID)
Harold Wiesenfeld, MD

SX-2 Application of Endolysins as Novel Antimicrobials Against Bacterial Vaginosis
Ryan Kean, PhD

SX-3 Novel *Eggerthellaceae* Isolates from the Female Reproductive Tract
Sujatha Srinivasan, PhD

1215 END OF WEDNESDAY GENERAL SESSIONS

1245-1400 YOUNG INVESTIGATOR'S PRESENTATIONS

Zingerman's Deli
422 Detroit St.
Ann Arbor, MI

Ann Arbor Farmers Market — Washtenaw County Farmers Markets. Wednesdays and Saturdays 7am–3pm

ANAEROBE 2024
July 8-11, 2024

Thursday, July 11

0730-0830 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS

0830-0930

SESSION XI: ADVANCES IN PUBLIC HEALTH/EPIDEMIOLOGY

Moderator: *Audrey Schuetz, MD*

SXI-1 Genomic Epidemiology-Providing Insights into Transmission and Spread of Pathogens at Multiple Spatial and Temporal Scales
Evan Snitkin, PhD

SXI-2 *Fusobacterium nucleatum*: A Model Periodontal Pathogen in Intrauterine Infections.
Yiping W. Han, PhD

SXI-3 Identification of Cryptic *Clostridioides spp*. among *Clostridioides difficile* Infection (CDI) Surveillance Isolates Collected through the Emerging Infections Program (EIP), 2018-2020
Giulia Orazi, PhD

0930-1030

SESSION XII: HOST RESPONSES IN ANAEROBIC INFECTIONS

Moderator: *Krishna Rao, MD*

SXII-1 Olfactomedin-4 + Neutrophils Exacerbate Intestinal Epithelial Damage and Worsen Host Survival after *Clostridioides difficile* Infection
Rajat Madan, MD, PhD

SXII-2 Loss of S100B Attenuates Severity of *Clostridioides difficile* Infection in Mice and Protects Enteric Glia
Deiziane Viana da Silva Costa, PhD

SXII-3 Toxin-Mediated Alterations in Host Physiology Impact *Clostridioides difficile* Colonization and Pathogenesis
Christopher Peritore-Galve, PhD

1030-1045 BREAK / INDUSTRY EXHIBITS

1045-1145

SESSION XIII: ANAEROBES IN THE ENVIRONMENT (NOVEL ANAEROBES)

Moderator: *Cynthia Sears, MD*

SXIII-1 Barcoded Overexpression Libraries to Study Gene Functions in Gut Bacteroidales
Yolanda Yue Huang, PhD

SXIII-2 Exploring the Role of Sodium Membrane Energetics in *Bacteroides fragilis* Bile Acid Resistance
Matthew Schnizlein, PhD

SXIII-3 The Role of Bile Acid Conjugation in Gut Inflammation
Yousi Fu, PhD

1145-1315 LUNCH / INDUSTRY EXHIBITS

1200-1300

SATELLITE SESSION

Introducing Cerillo: Flexible, Scalable, Highly Connected Tools for Studying Microbes in Any Environment
Julia Swavola, PhD

Thursday, July 11

1315-1415

SESSION XIV: *CLOSTRIDIOIDES DIFFICILE* II: BASIC RESEARCH

Moderator: *Aimee Shen, PhD*

SXIV-1 Novel Cell Wall Crosslinking Enzymes in *Clostridioides difficile* Produce Essential 3-3 Crosslinks
Kevin Bollinger

SXIV-2 Siderophore Utilization in *Clostridioides difficile*
Jessica Hastie, PhD

SXIV-3 Inhibition of *Clostridioides difficile* Sporulation by the Phosphotransfer Proteins PtpA and PtpB
Adrienne Edwards, PhD

1415-1430 BREAK / INDUSTRY EXHIBITS

1430-1530

SESSION XV: ANAEROBIC RESISTANCE AND NOVEL TREATMENTS

Moderator: *Vincent B. Young, MD, PhD*

SXV-1 The Implementation of FDA-Approved Microbiome Therapeutics: A Clinician Perspective for *Clostridioides difficile* Infections
Nirja Mehta, MD

SXV-2 Omics Evaluation of Omdacycline, A Low-Risk Antibiotic for *Clostridioides difficile* Infection
Jinhee Jo, PharmD

SXV-3 Beta-Lactamase as a Predictor of Penicillin Susceptibility for Anaerobic Gram-Negative Bacilli
Samantha Shannon, MLS (ASCP)CM

1530-1730 POSTER SESSION I / INDUSTRY EXHIBITS

1730 CONGRESS ADJOURNS

1745

CONGRESS RECEPTION / BANQUET / AWARDS

FINEGOLD AWARD

YOUNG INVESTIGATORS AWARD

LIFETIME ACHIEVEMENT AWARD—*Dr. Ellie JC Goldstein, MD*

FOUNDER'S AWARD

Main Street Ann Arbor Shopping & Dining

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

Monday, July 8 — Keynote Session

1800-1900 Session I: Keynote Presentation

*—Indicates Presenter

EVOLUTION OF THINKING ABOUT THE ROLE OF ANAEROBIC MICROBES IN GASTROINTESTINAL HEALTH AND DISEASE

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Research emphasis has evolved from concentrating on single pathogens to microbiota community structure and, more recently, to bacterial functions in defined micro-environments and *in vitro* and *in vivo* functional interactions between various bacteria. Furthermore, therapeutic approaches have transitioned from targeting pathogens with antibiotics to promoting colonization resistance and restoring healthy microbial metabolic function to promote homeostasis. Using examples from murine models of chronic immune-mediated experimental colitis and human inflammatory bowel diseases (IBD), I will discuss the power of using gnotobiotic techniques, human fecal transplant (FMT, primary and mouse-adapted) and functionally designed bacterial strain consortia to restore a healthy luminal environment to prevent and treat experimental colitis in humanized mice (germ-free mice colonized with single or pooled human fecal transplants). Snapshots of ongoing/recently completed studies will include mouse-adapted human IBD transplants to improve bacterial transfer efficiency and decreased phenotypic variability in transplant recipients, functional reciprocal interactions between mucus-digesting *Ruminococcus gnavus* and adherent-invasive *E. coli* or *Clostridium perfringens*, a novel functional immunologic high throughput screening method to identify protective vs aggressive resident bacterial strains in complex human bacterial strain consortia, use of a targeted bacteriophage consortia to selectively eliminate a pathobiont *in vivo*, and the effects of clinically relevant dietary proteins on microbial community structure, function and inflammatory potential. A vision of how these lessons from murine models might be applied to human IBD treatment will be shared, with the hope of adopting a more physiologic, less toxic therapeutic approach in contrast to current sustained immunosuppression. These lessons from IBD models have clear implications for preventing recurrent *Clostridioides difficile* infection and treatment of other consequences of dysbiosis (necrotizing enterocolitis, metabolic syndrome, MASH, etc.).

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Tuesday, July 9 — Anaerobes: Disease Associations

0830-0930 Session II: Anaerobes: Disease Associations

∗—Indicates Presenter

†Sponsored by European Society of Clinical Microbiology & Infectious Diseases (ESCMID)

ROLES OF THREE *FUSOBACTERIUM NUCLEATUM* SUPSPECIES IN COLORECTAL CANCER

Kuehne, S.A.;*†[1] Neo, O.;[2] Berditchevski, F.[3]
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A growing body of research has shown an association of certain oral bacteria with colorectal cancer (CRC). Of particular interest is the oral anaerobe *Fusobacterium nucleatum*, which has been found to be enriched in CRC tissues compared to controls.

This study investigated the adhesion and invasion dynamics of colorectal cancer (CaCo-2) cells by three subspecies of *F. nucleatum*: ssp. *animalis*, *nucleatum* and *polymorphum* and two adhesion mutants of ssp. *nucleatum*.

Methods included adhesion and invasion assays, transcriptomic analyses of the *F. nucleatum* subspecies to determine the gene expression profile at different growth phases and proteomic profiling of the inflammatory mediators secreted by CaCo-2 cells upon *F. nucleatum* subspecies infection.

F. nucleatum subspecies showed different rates of adhesion to CaCo-2 cells with ssp. *polymorphum* showing a statistically significant increase in adhesion compared to the others. Using deletion mutants, two major *F. nucleatum* adhesins Fap2 and FadA were found to be important for adhesion/invasion to Caco-2 cells. Furthermore, these experiments revealed that rate of adhesion was bacterial growth phase dependent, which was backed up by transcriptomic analysis, showing gene expression differences between stationary and exponential phase cultures.

Finally, a total of three cytokines were significantly differentially expressed out of 45 measured biomarkers, of which two (CXCL10 and IL-17C) were highly expressed in the *F. nucleatum* subspecies compared to a control. Whilst CXCL10 showed an increase in all subspecies, only subspecies *nucleatum* and *polymorphum* led to higher expression of IL-17C, which has been shown previously to aid in tumour promotion.

This study uncovered subspecies-specific differences in host-pathogen interactions, demonstrating the complex interplay of inflammatory mediators, host factors, pathogens and their characteristics in the tumour environment. Understanding the differences between *F. nucleatum* subspecies can uncover specific mechanisms which are key to their role in CRC.

†Sponsored by European Society of Clinical Microbiology & Infectious Diseases (ESCMID)

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CLOSTRIDIOIDES DIFFICILE INFECTION TRIGGERS LATE NEUROINFLAMMATORY RESPONSE IN THE BRAIN

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Previous studies have associated delirium and dementia with poor outcomes of *Clostridioides difficile* infection (CDI) in older adults. We examined how CDI may affect the brain in the mouse model of CDI. 6-month-old mice were infected with 10^5 CFU of *C. difficile*, monitored daily, and euthanized on day 3 and day 7 post-infection (pi). Prefrontal cortex, hippocampus, and cecum samples were analyzed for pro-inflammatory cytokine levels (S100B, IL-6, IL-1β, IL-13, IL-33, myeloperoxidase-MPO, TNF-α, KC, IL-17, IL-23, IL-10) by ELISA and for GFAP and phosphorylated NFκB (pNFκB) by western blot. Spearman correlation analyses were performed. Infected mice developed diarrhea and weight loss, resulting in maximum weight loss and diarrhea on day 3 pi, which were resolved by day 7 pi. Although inflammatory marker analysis still showed elevated levels of IL-1β, MPO, IL-13, IL-33, TNF-α, KC, IL-17, IL-23 and IL-10 in the cecum tissues from infected mice compared to uninfected mice on day 7 pi, most of these mediators (except for IL-23, IL-13, MPO and IL-33) exhibited much higher levels in infected cecum tissues on day 3 pi. In the prefrontal cortex and hippocampus, no alterations in any of these cytokines were detected on day 3 pi when comparing the infected to the uninfected group. However, on day 7 pi, levels of hippocampal S100B ($p=0.003$) and IL-6 ($p= 0.03$), as well as prefrontal cortex levels of IL-13 ($p=0.05$), IL-33 ($p=0.03$), and MPO ($p=0.04$) were elevated in infected mice on day 7pi. Prefrontal cortex IL-33 levels on day 7 pi were found to be positively correlated with both the clinical scores and the cecal IL-33 levels of the mice during the peak of infection (day 3 pi). Moreover, prefrontal cortex levels of GFAP were increased and hippocampus levels of pNFκB were detectable on day 7 pi in the infected mice, pointing to reactive astrocytes and an NFκB pathway as a mechanism by which the neuroinflammation is induced post-CDI. Our findings demonstrate that CDI promotes inflammation in the prefrontal cortex and hippocampus which occurs after the peak of intestinal inflammation. IL-33, MPO and IL-6 appear to be involved in the neuroinflammatory effect associated with CDI.

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INVESTIGATING THE ROLE OF THE GUT MICROBIOTA IN THE 3KL MODEL OF PARKINSON'S DISEASE USING SELECTIVE ANTIBIOTIC DEPLETION.

Beauchamp, L.C.;* Li, S.; O'Brien, M.; Ekwudo, M.; Nuber, S.; Weiner, H.L.; Cox, L.M.
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The gut microbiota may contribute to motor dysfunction in Parkinson's disease; however, the contribution of specific bacteria is not fully understood. To identify changes in the gut microbiota associated with disease progression in the 3KL model of PD, we utilized individual antibiotics to deplete specific bacterial populations. The 3KL mouse is a model of PD, which expresses mutant alpha-synuclein (aS). This mutation results in a loss of the physiologic tetramer:monomer ratio of aS, resulting in pathologic aggregation, and eventual development of motor deficits that respond to levodopa. We administered metronidazole, neomycin, or penicillin to male and female 3KL mice from 7-12 months of age, then tested motor function over time. We performed 16S rRNA sequencing on longitudinal stool samples and characterized microglia responses by RNA sequencing. Metronidazole, an antibiotic that targets Gram negative anaerobic bacteria, slowed the progression of the motor phenotype as measured by hindlimb clasping in male 3KL mice, but not in female mice. Neomycin and penicillin did not affect motor function. Using 16S sequencing, we identified specific bacteria in the microbiome depleted by metronidazole but not depleted by neomycin or penicillin. In microglia, metronidazole decreased expression of genes in multiple KEGG pathways associated with infections, including Salmonella, Pertussis, Legionellosis, and Measles, whereas metronidazole increased expression of microglial genes involved in the lysosome, oxidative phosphorylation, and protein processing in the endoplasmic reticulum. These data suggest that metronidazole-sensitive bacteria contribute to motor deficits in a sex-specific manner, which is linked to suppressing inflammatory microglial responses associated with infection and increasing microglial gene expression related to protein degradation.

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Tuesday, July 9 — Anaerobic Infections

0930-1030 Session III: Anaerobic Infections

*—Indicates Presenter

†Sponsored by European Society of Clinical Microbiology & Infectious Diseases (ESCMID)

FINDING *FUSOBACTERIUM NECROPHORUM:* A JOURNEY OF PATIENT AND PATHOGEN DISCOVERY

Perry, M.*†
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Fusobacterium necrophorum is a human pathogen responsible for an array of clinical entities ranging from acute pharyngitis to Lemierre's syndrome. An overview of the various clinical presentations commonly seen with *F. necrophorum* will be presented, with an accompanying Lemierre's patient story. Phenotypic/genotypic antimicrobial susceptibility data, whole genome sequencing virulence determinant detection and phylogenetic findings, from a collection of historic and contemporary *F. necrophorum* strains, will also be discussed.

†Sponsored by European Society of Clinical Microbiology & Infectious Diseases (ESCMID)

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FUSOBACTERIUM NUCLEATUM IS ENRICHED IN INVASIVE COLORECTAL CANCER BIOFILMS

Queen, J.R.;*[1] Drewes, J.L.;[1] Cing, Z.;[2] White, J.R.;[3] McMann, M.;[1] Nandi, A.;[1] Minsky, H.;[1] Southward, T.M.G.;[1] Ferri, J.;[1] Iyadorai, T.;[4] Vadivelu, J.;[4] Roslani, A.;[4] Loke, M.F.;[4] Wanyiri, J.;[4] Sears, C.L.[1]
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We characterized *Fusobacterium nucleatum* (*Fn*) and its subspecies in the tumor microbiome of a large colorectal cancer (CRC) cohort through a combination of molecular, microscopic, and microbiologic analyses, and integration of clinical and pathologic features.

The study population consisted of 110 CRC patients in Malaysia, from whom paired tumor and distant normal colon tissues were surgically biopsied. Demographic and clinical data collected included age, sex, ethnicity, tumor location, and tumor stage. Carnoy's fixed tissues were stained by multiprobe FISH to evaluate biofilms. Biofilms were prevalent on colon tumors, more often on right-sided tumors than left ($p<0.0001$). We visualized *Fusobacterium* in 74% of polymicrobial biofilms, often in dense blooms, and with a significant association with later tumor stage ($p<0.0001$). V3-V4 16S rRNA amplicon sequencing was performed on snap-frozen tissues, revealing that *Fn* was enriched in the tumor microbiome compared to paired normal samples ($p<0.0001$), but only in biofilm-positive tumors ($p<0.0001$), and at later cancer stages ($p=0.0012$, 0.0067, and <0.0001, respectively, for stages II-IV). Other oral organisms, *Gemella morbillorum* and *Parvimonas micra,* were positively associated with *Fn* in the tumor microbiome ($p=0.0008$, <0.0001). Using PCR to resolve the *Fn* subspecies, *animalis* was most frequently detected in the tumor microbiome (52% of *Fn*+ tumors), with a trend toward association with biofilm status (n=33 of 110 tumors assessed to date). *Fusobacterium* strains were isolated from tumor biopsies using selective culture media. Correlating with our PCR analysis, 50% of *Fn* strains isolated to date are subspecies *animalis*.

In conclusion, *F. nucleatum*, and specifically subsp. *animalis,* is enriched in the colon microenvironment in association with other oral microbiome members in tumor biofilms, particularly in advanced cancer stages. Our findings suggest that *F. nucleatum*:bacterial interactions within invasive biofilms is an understudied feature of CRC carcinogenesis.

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STRATEGIC DRUG REPURPOSING FOR BIOFILM ASSOCIATED BACTERIAL VAGINOSIS (BV)

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Purpose: To repurpose existing antimicrobials for use against *Gardnerella vaginalis* and associated multi-species biofilms representative of bacterial vaginosis (BV).

Methods and results: Two drug repurposing libraries; the global health priority box (n=240 compounds) and the pandemic response box (n=400 compounds) from the medicines for malaria venture (MMV) and drugs for neglected disease initiative (DNDi) were screened against planktonic *G. vaginalis* ATCC 14018 – a facultative anaerobe involved in BV onset and progression. From 640 compounds, we have demonstrated that 28 compounds were able to significantly inhibit (>90%) the growth of *G. vaginalis*. Of these, 10 compounds also inhibited *Lactobacillus crispatus* CCUG 42898, a beneficial commensal bacterium in the vaginal microenvironment, and were thus excluded from subsequent analysis. The remaining 18 compounds were streamlined according to potency and cytotoxicity to leave 10 strong candidates for further testing. These included previously developed antifungals and antibacterials at all stages of drug development (i.e. from preclinical development through to routine clinical use). Through further testing, all selected compounds were able to reduce the bioburden of *G. vaginalis* biofilms to varying degrees. Expanding this work into a previously developed multi-species anaerobic biofilm model representative of BV which comprises *G. vaginalis, Fannyhessea vaginae, Mobiluncus curtisii* and *Prevotella bivia,* we demonstrate significant compositional shifts following application of three candidates.

Conclusions: In this work, we have identified existing antimicrobials that are suitable for repurposing in bacterial vaginosis. These compounds showed significant and selective inhibition of *G. vaginalis* growth when compared with *L. crispatus.* Additionally, at the tested concentrations these compounds displayed no cytotoxic effect against HepG2 mammalian cells, and showed efficacy against *G. vaginalis* mono- and multi-species biofilms. Current work is ongoing, investigating these compounds against antibiotic-resistant clinical isolates and transcriptomics to unravel the cellular targets of these compounds.

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Tuesday, July 9 — Anaerobic Clinical Infections

1045-1145 Session IV: Anaerobic Clinical Infections

∗—Indicates Presenter

DECREASING NEEDLESS "SELF-FLAGYLLATION": OVER-USE OF METRONIDAZOLE IN ASPIRATION PNEUMONIA

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Aspiration pneumonia (Asp PNA) is an imprecise term applied to a recognized clinical entity often resulting from altered consciousness and associated with pulmonary injury secondary to inhalation of oropharyngeal or gastric secretions that contain pathogenic bacteria. In 1974, Bartlett, Gorbach and Finegold [AJM 1974;56:202] studied 54 patients with Asp PNA at the Los Angeles VA Hospital (transtracheal aspiration of sputum). They found 50/54 (93%) grew anaerobes which were the only pathogens in 25/54 (46%) of cases. Predominant anaerobes isolated were *Bacteroides melaninogenicus*, *Fusobacterium nucleatum*, anerobic or micro-aerophilic Gram-positive cocci, and "*Bacteroides fragilis*" which was found in 9/54 (17 %) patients. These findings resulted in the inclusion of anti-anaerobe antibiotics as part of the standard of care.

However, updated guidelines and expert opinion have shifted away from including dedicated anaerobic coverage for Asp PNA, such as the specific inclusion of metronidazole. This change is predicated on 1) updates in anaerobic methodology and taxonomy 2) the likely negligible contribution of *Bacteroides fragilis* to aspiration pneumonia and 3) the underappreciated activity of ceftriaxone and other agents against respiratory anaerobes.

Bartlett himself noted (CID 1993;16:(Suppl 4): S252) that reidentification of those *B. fragilis* isolates show" they were probably other penicillin-resistant *Bacteroides*."

Commonly used antibiotics for Asp PNA such as ceftriaxone, linezolid and others have underappreciated anaerobic activity against the common anaerobic species of Asp PNA (Data to be presented). Therefore, it is NOT that anaerobes are unimportant or infrequent pathogens, but are unappreciated by most studies. Respiratory anaerobes are and were treated with agents as ceftriaxone, negating the need for additional agents as metronidazole. Additionally, metronidazole has some potential toxicities, such as vomiting, diarrhea, neuropathy, and the promotion of resistant pathogen growth.

We posit that metronidazole is redundant for Asp PNA. However, future Asp PNA studies should evaluate the unappreciated anti-anaerobic activity of the specific agent(s) being tested.

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SERUM AND STOOL INFLAMMATORY MEDIATORS ARE VIABLE INDICATORS OF RISK FOR DEVELOPING RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Statement of purpose: We show the feasibility of serum and stool biomarker-based models as tools for identifying patients at risk of recurrent *Clostridium difficile* infection (CDI).

Background: CDI is a significant cause of morbidity/mortality with ~20% of patients experiencing recurrent CDI (rCDI). We are unable to accurately identify patients who are at risk for developing subsequent rCDI. In this observational study, we use serum and stool biomarkers at initial diagnosis to predict subsequent rCDI.

Methods: The retrospective cohort included 186 patients with diarrhea diagnosed with CDI by clinical stool test for toxigenic *C. difficile.* We defined rCDI as second episode within 100 days. Stool and sera were obtained per patient, and we measured the concentrations of 17 inflammatory mediators using a Luminex 200® instrument. Stool toxin activity was quantified by real-time, cell culture-based cytotoxicity assay using the xCelligence® instrument. Analysis included logistic regression and elastic net regression modeling with 5-fold cross validation across 100 iterations and optimal model selection by AUROC. Where relevant, stool inflammatory mediator and toxin levels were modeled as an interaction.

Results: Death and rCDI occurred in 32 (17%) and 36 (19%) patients, respectively. In unadjusted analysis, serum PCT, EGF, RANTES, IL-6, and IL-2Ra were associated with recurrence ($P<0.001$, $P=0.015$, $P=0.017$, $P=0.025$, and $P=0.035$ respectively). No stool biomarkers were individually associated with recurrence. Parsimonious elastic net modeling showed reduced performance of stool only models compared to serum only models for the prediction of recurrence with AUC 0.66 [0.54-0.78] (IP-10 and IL-15) and AUC 0.76 [0.69-0.84] (TNF-alpha, RANTES, PCT, CXCL-9, CXCL-5, HGF, and EGF), respectively. Combined serum-stool biomarker models showed no improvement with AUC 0.77 [0.68-0.86].

Conclusions: Serum biomarkers obtained during initial CDI episode are viable indicators for risk of CDI recurrence. Stool only models perform poorly compared to serum only models and provide no additional predictive power to combined models.

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COMPARING MORTALITY OF SEPSIS PATIENTS ADMINISTERED PIPERACILLIN-TAZOBACTAM OR CEFEPIME USING INSTRUMENTAL VARIABLE ANALYSIS

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Importance: Bacterial sepsis is a leading cause of mortality among hospitalized patients. Effective treatment requires early administration of antibiotics, which are often selected empirically. Recent studies have suggested that treatment with anti-anaerobic antibiotics (such as piperacillin-tazobactam) is associated with adverse clinical outcomes, compared to treatment with anaerobe-sparing antibiotics (such as cefepime).

Objectives: Determine the impact of anti-anaerobic piperacillin-tazobactam compared to anaerobe sparing cefepime on 90-day mortality in patients treated empirically for sepsis using instrumental variable analysis of a 15-month piperacillin-tazobactam shortage.

Design: Retrospective cohort study.

Setting: Hospital admissions at the University of Michigan from July 2014 to December 2018, including a piperacillin-tazobactam shortage period from June 2015 to September 2016.

Participants: Adult patients with suspected sepsis treated with piperacillin-tazobactam or cefepime for conditions with presumed equipoise between the two agents.

Main Outcomes and Measures: The primary outcome was 90-day mortality. The 15-month piperacillin-tazobactam shortage period was used as an instrumental variable for unmeasured confounding in antibiotic selection.

Results: Among 7,569 patients with sepsis meeting study eligibility, 4,523 were treated with piperacillin-tazobactam and 3,046 were treated with cefepime. Of patients who received piperacillin-tazobactam, only 152 (3%) received it during the shortage. Treatment groups did not differ significantly in age, medical comorbidities, acuity of illness, or time to antibiotic administration. In an instrumental variable analysis, piperacillin-tazobactam was associated with an absolute mortality increase of 5.0% at 90 days (95% CI 1.9-8.5%).

Conclusions and Relevance: Among patients with suspected sepsis and no clear indication for anti-anaerobic coverage, administration of piperacillin-tazobactam was associated with higher mortality and increased duration of organ dysfunction as compared to cefepime. These findings suggest that the widespread use of empiric anti-anaerobic antibiotics in sepsis may be harmful.

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Tuesday, July 9 — Health & Disease

1315-1415 Session V: Anaerobes in Health & Disease

∗—Indicates Presenter

DELETED IN MALIGNANT BRAIN TUMORS 1 GLYCOPROTEIN IS LOST IN COLONIC DYSPLASIA

Green, E.H.;[1] Kotrannavar, S.R.;[1] Rutherford, M.E.;[2] Kaur, H.;[1] Lunnemann, H.M.;[2] Heiser, C.N.;[1] Wu, S.;[3] Ding, H.;[3] Simmons, J.A.;[1] Liu, X.;[2] Lacy, D.B.;[2,4] Shrubsole, M.J.;[2] Liu, Q.;[2] Lau, K.S.;[1] Sears, C.L.;[3] Coffey, R.J.;[2] Drewes, J.L.;[3] Markham, N.O.*[2,4]
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[2]Vanderbilt University Medical Center, Nashville, TN USA
[3]Johns Hopkins University, Baltimore, MD USA
[4]Department of Veterans Affairs, Nashville, TN USA

Colorectal cancer (CRC) is responsible for 50,000 deaths annually in the United States. Emerging evidence supports a causal role for pro-carcinogenic bacteria in the colonic microbiome. We previously showed toxigenic *C. difficile* from human CRC-associated bacterial biofilms accelerates tumorigenesis in *Apc*$^{Min/+}$ mice. To understand host-microbe interactions during colonic tumorigenesis, we combined single-cell RNA-sequencing (scRNA-seq), spatial transcriptomics, and immunofluorescence to define the molecular spatial organization of colonic dysplasia. We analyzed two groups of gnotobiotic *Apc*$^{Min/+}$ mice: one group colonized with a 30-bacteria consortium, including *C. difficile*, isolated from a human CRC-associated biofilm, and a second group colonized with 29 bacteria, lacking *C. difficile*. From scRNA-seq, differential gene expression analysis of absorptive colonocytes showed the glycoprotein Deleted in Malignant Brain Tumors 1 (DMBT1) is upregulated by *C. difficile* compared to colonocytes from mice without *C. difficile* exposure. Surprisingly, our spatial transcriptomic analysis showed DMBT1 was dramatically downregulated in dysplastic foci compared with normal-adjacent tissue. We show DMBT1 protein is downregulated in dysplastic foci from 3 mouse models of colonic tumorigenesis. We confirmed this pattern of DMBT1 downregulation in human dysplasia compared with normal-adjacent crypts. Using spatial transcriptomics from human CRC and adenomas, we found DMBT1 downregulated in most dysplasia but its expression appeared increased in some regions concomitantly with interferon-stimulated genes. We further integrated our datasets to generate custom spatial deconvolution, ligand-receptor mapping, and colonic dysplasia scores. Together, our data reveal cell type-specific regulation of DMBT1, a potential mechanistic link between bacteria and colonic tumorigenesis.

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IN SILICO DESIGN OF BACTERIAL CONSORTIA MITIGATES *CLOSTRIDIOIDES DIFFICILE* PATHOGENESIS IN A MURINE MODEL

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Fecal Microbiota Transplant (FMT) is an emerging therapy that has had remarkable success in treatment and prevention of recurrent *Clostridioides difficile* infection (rCDI). FMT has recently been associated with adverse outcomes including inadvertent transfer of antimicrobial resistance or pathogens, thereby necessitating development of more targeted bacteriotherapies. To address this challenge, we developed a novel systems biology pipeline to identify candidate bacterial strains predicted to mitigate *C. difficile* pathogenesis through alteration of bacterial metabolism. To do this, metagenomic characterization of human FMT donor samples were used to identify the top metabolic pathways associated with successful FMTs (defined as no recurrence within three months after receiving FMT). Metabolic models of the encoding species were reconstructed to simulate competitive or cooperative metabolic interactions with *C. difficile* (R20291, iCdR703). Results from metabolic interaction predictions were used to assemble different consortia of bacteria to test *in vitro*. Notably, consortia containing four species that either cooperated or competed for metabolites based on active exchange reactions were tested *in vitro*. More specifically, competitive or cooperative consortia were grown to saturation in rich media and then the resultant 'spent' media used to grow *C. difficile* in. *In vitro* results show spent media from a cooperative community enables growth of *C. difficile* suggesting a cooperative exchange and utilization of metabolites. Conversely, competition for nutrients from the competitive community resulted in reduced growth. We subsequently tested the two consortia in a murine model of CDI, demonstrating amelioration from severe CDI through decreased toxin levels and diversification of the intestinal microbiome when comparing cooperative versus the competitive community or control. Importantly, we conclude that the predictive platform based on *in silico* and *in vitro* metabolic interactions between the microbiota and *C. difficile* led to a rationally designed biotherapeutic framework that may be extended to infections that involve alteration of the microbiome.

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NOMMS: THE NASAL AND ORAL MICROBIOME IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an autoimmune disease that may be affected by the mucosal microbiota at different anatomical sites. While changes in the gut microbiota have been linked to MS, little is known about the nasal and oral microbiota, which may also be in contact with the mucosal immune system. The gingival microbiota has been shown to drive systemic inflammation, pathogens within the pharyngeal microbiota can lead to autoimmune-mediated neurologic diseases, and altered bacterial toxigenic genes have been found in active MS.

In this project, nasal and oral swabs were collected from multiple sclerosis subjects and healthy volunteers to compare the microbial compositions across 3 anatomical sites ranging nostril, gums, and throat. We profiled the microbial composition using 16S phylogenetics and identified site-specific bacteria. Linear regression results showed several common or rare opportunistic bacteria were correlated with disease worsening in the context of multiple sclerosis, while controlling for confounding factors such as smoking, oral health, vitamin D uptake, and other subject demographics. Overall, our NOMMS data suggested that mucosal bacteria closely related to MS disease status and can be used as a potential probe to indicate MS disease progression.

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Tuesday, July 9 *C. difficile I:* Clinical & Epidemiology Updates

1430-1530 Session VI: *Clostridioides difficile I:* Clinical & Epidemiology Updates

*—Indicates Presenter

EVOLUTION OF THE TREATMENT OF CDI—FROM METRONIDAZOLE TO FDA-APPROVED FMT

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The epidemiology of *Clostridioides difficile* infection (CDI) has changed dramatically, since we first learned of the causative organism in the 1970s. The most prominent changes occurred during the epidemic in the early 2000s, driven by the NAP1/BI/ribotype 027 strains that circulated first in North America but then quickly spread worldwide. Suddenly, many more patients were developing CDI and experiencing both severe and recurrent disease, the latter of which to-date remains a recalcitrant problem for many and fuels the ongoing morbidity and cost of CDI today. This epidemic spurred innovation in CDI diagnostics and therapeutics—we have gone from a paucity of sensitive, rapid diagnostic tools to many rapid molecular tests available to clinicians. However, the pendulum has swung in the other direction—many current tests are so sensitive they frequently cause false positive results among colonized patients, prompting recent and ongoing adjustments to how CDI is tracked by the CDC's National Health Safety Network. The molecular epidemiology of CDI has changed with epidemic ribotypes becoming less prominent and community-acquired CDI rising in incidence, and it is clear that *C. difficile* spores are frequently encountered in daily life. Thus, we have learned about the importance of minimizing high risk antibiotic and proton pump inhibitor exposures both in and out of the hospital to prevent initial and recurrent CDI. We have learned about the relative importance of community-acquired vs. intra-facility spread of CDI and implications for infection prevention practices. An understanding of the biology of CDI and its interaction with the host and gut microbiome has enabled the rational design of treatments, and we now have new antimicrobials, a monoclonal antibody, and live biotherapeutics that are FDA-approved to treat CDI and prevent recurrence, with holistic approaches that include dietary fermented foods also showing promise. Future progress in the field requires excellent diagnostic stewardship, accurate prognostic information to guide treatment decisions, and affordable, holistic, and science-based treatment plans that effectively treat initial CDI and prevent recurrence.

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MICROBIOME EVALUATION FROM THE PHASE 2, RANDOMIZED, DOUBLE-BLIND STUDY OF IBEZAPOLSTAT COMPARED WITH VANCOMYCIN FOR THE TREATMENT OF *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Background. Ibezapolstat (IBZ) is Gram-positive selective spectrum antibiotic that inhibits the bacterial DNA polymerase IIIC currently in clinical trial development for the treatment of *C. difficile* infection (CDI) in adults. In the open-label, non-comparative, phase 2a study, 10 of 10 IBZ-treated CDI patients experienced clinical cure. The purpose of the phase 2b study was to assess microbiome differences between IBZ versus vancomycin (VAN) for treatment of CDI.

Methods. Phase 2b was a randomized, double-blind, active-comparator study. Participants with signs and symptoms of CDI and a positive enzyme immunoassay toxin test result were recruited from 12 centers in the USA and randomly assigned (1:1) to receive oral IBZ 450 mg every 12 h or oral VAN 125 mg every 6 h for 10 days. Stool was collected daily for microbiome evaluations (qPCR and metagenomic changes). The trial is registered with ClinicalTrials.gov, number NCT04247542.

Results. Thirty patients were recruited and were assessed in the per protocol analysis (IBZ: n=16; VAN n=14). Fifteen of 16 (93.8%) patients given IBZ (one clinical failure) had a sustained clinical cure versus 12 of 14 (86%) patients given VAN (two recurrences). IBZ patients had stable or increased concentrations of Bacteroides, *C. leptum* and *C. coccoides* without an increase in Enterobacterales. Decreased concentrations of these microbiome species were observed in VAN-treated patients, especially noted in the two patients with CDI recurrence.

Conclusions. In the phase 2b study, IBZ had a favorable microbiome effect increasing concentrations of *C. leptum* and other beneficial commensals while on therapy. Patients with CDI recurrence on VAN had significant disruption to the microbiome. These results warrant further development in phase 3 trials.

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MOLECULAR EPIDEMIOLOGY OF *CLOSTRIDIOIDES DIFFICILE* IN THE UNITED STATES, 2020

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The Centers for Disease Control and Prevention (CDC) performs *Clostridioides difficile* infection (CDI) surveillance, including isolate characterization, through the Emerging Infections Program (EIP) to describe changes in strain prevalence over time. We present the molecular epidemiology of *C. difficile* isolates collected in the United States in 2020.

A convenience sample of *C. difficile* isolates from 10 EIP sites (CA, CO, CT, GA, MD, MN, NM, NY, OR, TN) were sequenced (Illumina MiSeq, NextSeq, and NovaSeq) by CDC or the MN Department of Health Public Health Laboratory. Assembly and multi-locus sequence type (ST) analyses were performed using CDC's in-house PHoeNIx pipeline. An in-house ST-to-ribotype (RT) crosswalk was used to infer RTs.

In 2020, 1007 isolates underwent whole genome sequencing (WGS). Overall, 102 STs were identified. Of 510 healthcare-associated (HA) isolates, 70 STs were observed and ST42 was predominant (66/510, 13%), followed by ST8 (11%), ST1 (11%), ST2 (9%), and ST53 (6%); all other STs consisted of <5% of isolates. ST42 was most prevalent among HA isolates in 3 EIP sites (CA, CO, TN). Of 497 community-associated (CA) isolates, 72 STs were observed and ST42 (60/497, 12%) and ST2 (59/497, 12%) were predominant, followed by ST8 (9%), and ST1 (5%); all other STs consisted of <5% of isolates. ST42 was the most prevalent CA ST in 5 EIP sites (CO, CT, NM, OR, TN).

In 2020, ST42 remained the predominant HA and CA ST, with no change compared to 2019. Isolates with ST42 (inferred RT106) have been associated with increased antibiotic resistance, spore production, and potential to cause recurrent disease. Compared to 2019, there was no change in the percentage of HA and CA isolates with ST1, which is inferred to be the epidemic RT027 strain type.

Historically, ST1/RT027 was the predominant strain type, but recently has been replaced by a distribution of different STs. Continued surveillance is necessary to monitor trends; future work will utilize WGS data for analyses of toxin and antimicrobial resistance genes.

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Wedensday, July 10 — Microbe Interactions

0800-0900 Session VII: Interspecies & Microbe/ Microbe Interactions

∗—Indicates Presenter

BIOACTIVE COMPOUNDS PRODUCED BY THE GUT COMMENSAL *ENTEROCLOSTER CITRONIAE* MODULATE ENTERIC PATHOGEN VIRULENCE

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Our group studies the role of small molecules in host-microbiota-pathogen interactions. We have, thus far, focused on the impact of microbiome-derived compounds on enteric pathogen behavior. Using untargeted metabolomics, we previously showed that the human gut harbors thousands of small molecules, most of which are unknown. The gut microbiome is directly or indirectly involved in the production of the majority of these compounds, as an antibiotic treatment regimen that eliminates 90% of gut bacteria resulted in altered levels of 80% of the metabolite features detected. We then hypothesized that some of these compounds may elicit responses in various cell types present in the gut environment. Organic extracts of human feces were then used to determine transcriptional responses of various pathogens to the chemical milieu of the human gut through mRNA sequencing. Our results showed that multiple enteric pathogens, such as *Vibrio cholerae*, *Salmonella enterica*, and *Clostridioides difficile* display marked transcriptional responses to the human gut metabolome, and that genes required for host interactions are often modulated. In the case of *V. cholerae*, we showed that both toxin production and swimming motility are drastically repressed during bacterial growth in the presence of fecal extracts. Also, we were able to isolate bioactive members of the gut microbiome; when grown in pure cultures, *Enterocloster citroniae* produces bioactive compounds that repress *V. cholerae* swimming motility, recapitulating the effect of the full fecal extract. Host cell interaction assays using cultured colonic epithelial cells showed that small molecules produced by *E. citroniae* modulate toxin-mediated host cell death induced by *V. cholerae*. Previously, we have been able to identify microbiome-derived bioactive compounds that affect *S. enterica* virulence, the most active of which was 3,4-dimethylbenzoic acid (DMB). Interestingly, DMB is not responsible for the effect on *V. cholerae*, suggesting that a new bioactive compound is involved. Ongoing work is focused on identifying the bioactive compound produced by *E. citroniae* and revealing the molecular mechanisms behind bioactivity.

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PROTECTION AGAINST CDI COLITIS IN A MOUSE MODEL BY A CLINICAL AVIRULENT *CLOSTRIDIOIDES DIFFICILE* ISOLATE THROUGH AMINO ACIDS COMPETITION

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Clostridioides difficile strains belonging to the epidemic BI/NAP/027 group have been associated with increased transmissibility and disease severity. While these strains encode multiple toxins, the major toxin A and toxin B virulence factors and the CDT binary toxin, not all BI/NAP/027 strains cause disease. We previously isolated an avirulent *C. difficile* RT027 strain, ST1-75, from a patient-derived fecal sample that does not cause diarrhea or morbidity in antibiotic-treated mice, despite colonizing the colon and producing fecal toxins comparable to highly virulent RT027 strains. Surprisingly, concurrent infection of mice with ST1-75 and virulent *C. difficile* strains prevents the development of colitis because the avirulent strain rapidly out-competes the virulent strains. The ability of ST1-75 to protect against acute colitis was not due to phage activation or a mutation in the *cdtR* gene, which encodes a response regulator that activates expression of the gene encoding binary toxin/CDT. Instead, the avirulent ST1-75 out-competes virulent *C. difficile* strains, because it has a greater capacity to deplete amino acid pools during infection in mice. These data reveal a mechanism for mediating colonization resistance between strains of the same ribotype (RT027) and demonstrate that ST1-75 is a promising biotherapeutic candidate for use in CDI prevention for high-risk patients.

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FUSOBACTERIUM NUCLEATUM AND *CLOSTRIDIOIDES DIFFICILE* CO-COLONIZATION INCREASES INFLAMMATION OF THE GUT EPITHELIUM

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Inflammatory bowel disease (IBD) is a chronic condition affecting millions of individuals worldwide. IBD is characterized by uncontrolled intestinal inflammation affecting a patient's quality of life. IBD patients experience significant shifts in their intestinal bacterial communities including colonization by the oral microbe *Fusobacterium nucleatum*. Additionally, IBD patients have an elevated risk of being infected by the pathogen *Clostridioides difficile*. Unfortunately, IBD patients with *C. difficile* experience more severe clinical outcomes than non-IBD patients. The association between IBD and *C. difficile* may be due to a variety of factors, but one hypothesis is that the altered gut microbiota of IBD patients may contribute to worse infection. We hypothesized that *F. nucleatum* works cooperatively with *C. difficile* to promote inflammation and exacerbate colitis. *In vitro,* we found that *C. difficile* and *F. nucleatum* grew well together in a chemically defined medium and grew on diverse nutrient sources in Biolog Phenotypic microarrays when grown together. Additionally, we found that human colonic epithelial cells treated with *F. nucleatum* and *C. difficile* exhibited elevated expression of pro-inflammatory cytokines IL-8 and TNF compared to cells with *C. difficile* alone. To address if *F. nucleatum* could worsen *C. difficile* infection, we utilized a mouse model of *C. difficile*. C57B6/J mice were pretreated with a cocktail of antibiotics and Clindamycin then followed with a single oral gavage of a PBS vehicle control, *C. difficile* R20291, *F. nucleatum*, or a combination of *C. difficile* and *F. nucleatum*. As expected, our PBS group alone did not exhibit signs of inflammation. Our *F. nucleatum* and *C. difficile* mono-treated mice experienced low and moderate inflammation respectively. However, our mice treated with *C. difficile* and *F. nucleatum* exhibited extensive immune inflammation and overall higher histological scores. Our data supports that *F. nucleatum* and *C. difficile* have a synergistic relationship and stimulate robust inflammatory responses highlighting the importance of understanding bacterial interactions in the context of infection.

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0900-1000 Session VIII: Other Anaerobes: Physiology & Clinical Associations

∗—Indicates Presenter

ANALYZING THE *TREPONEMA DENTICOLA* PROTEASE COMPLEX (DENTILISIN) IN CLINICAL ISOLATES

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Objectives: *Treponema denticola* is one of several "keystone" periodontal pathogens. Dentilisin, its surface-localized protease complex, contributes to the dysregulation of tissue homeostasis driving the disease process. While protease activity (encoded in PrtP) is well-characterized, mechanisms by which the 3 lipoproteins comprising dentilisin interact to form a stable outer membrane complex are unknown. To identify its potential interacting domains, we examined *T. denticola* clinical isolates for sequence variations in the proteins comprising dentilisin (PrcB, PrcA and PrtP) and generated a predictive model of the dentilisin complex.

Methods: We are sequencing *prcB, prcA* and *prtP* amplicons in >60 *T. denticola* clinical isolates. Isolates were first grouped by variations in PrcB and the PrtP C-terminal domain. We are currently amplifying and sequencing the remainder of the dentilisin locus (5' region of *prcB* through 3' region of *prtP*) in these strains. Molecular models of PrcB, PrcA and PrtP were generated using the D-I-TASSER and AlphaFold algorithms.

Results: The PrtP C-terminus fell into 11 groups reflecting considerable interstrain amino acid sequence variability, consistent with its predicted extracellular exposure and antigenicity. Both PrcA and the PrtP N-terminus including the subtilisin-like proteolytic domain were generally well conserved. PrcB sequences were very highly conserved (88% identity) in all strains. We identified 11 PrcB groups, plus 4 strains that did not fall in any group. Four PrcB groups had identical PrcB and PrtP sequences. Preliminary models of the dentilisin complex generated in AlphaFold show the predicted interacting regions of PrcA, PrcB and PrtP. Interestingly, the PrtP C-terminal domain interacts with neither PrcA nor PrcB.

Conclusions: Our results support the hypothesis that the PrtP C-terminal domain contains a surface-exposed antigenic domain distinct from its conserved proteolytic domain. High conservation of PrcB sequences is consistent with the requirement of PrcB for expression of PrtP protein. This study is part of a larger project including molecular modeling of the dentilisin complex and protein-protein interaction studies.

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BACTEROIDES THETAIOTAOMICRON PREVENTS DEATH FROM *CLOSTRIDIOIDES DIFFICILE* INFECTION IN AGED HOST: POTENTIAL ROLE OF INTERFERON SIGNALING

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Purpose: *Clostridioides difficile* infection (CDI) leads to more severe disease in the older patients than younger patients. Aged mouse model of CDI showed that older age leads to more deaths as well as altered immune response. When fecal microbiota transplant (FMT) from young mice was given to aged mice, aged mice were protected from death. In the current study, we aim to study the mechanisms of protection by which microbiota can protect aged host against death from CDI.

Methods and Results: Aged mouse model of CDI was used to test the effect of microbiome intervention such as FMT or administration of bacteria isolated from young mouse stool. Metabolomics, RNA sequencing of tissue, and flow cytometry of immune cells in intestinal tissue were performed to elucidate the mechanism of protection.

FMT was not consistently effective in protecting aged mice against death from CDI, and the protection correlated with higher number in the Bacteroidetes phylum. Utilizing Bacteroides-selective medium, *Bacteroides thetaiotaomicron* (BT) was isolated from young mouse stool. Treatment with BT showed protection against CDI equivalent to FMT. Metabolomics showed that secondary bile acid was increased and primary bile acid decreased with BT treatment, which is consistent with the changes seen with FMT. Flow cytometric analysis did not reveal a significant change in immune cell such as Th cells, Treg cells, or innate lymphoid cells. Utilizing RNA seq, we determined that pathways such as interferon-alpha and interferon-gamma were increased with treatment with BT.

B. thetaiotaomicron emerged as a key bacterial specie conferring protection against CDI in aged host in the mouse model. Change in bile acids and corresponding change in host immune response pathways suggest interaction between these two components. Involvement of interferon pathways in immune response against bacteria is a novel finding, consistent with previous findings on the effect of microbiome on antiviral immune response.

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ANTIMICROBIAL SUSCEPTIBILITY OF GRAM NEGATIVE ANAEROBIC ISOLATES IN A HIGH COMPLEXITY PEDIATRIC HOSPITAL FROM BUENOS AIRES, ARGENTINA

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Routine antimicrobial testing of anaerobes in reference-level laboratories is recommended to monitor local susceptibility and guide appropriate empiric treatment. The aim of this study was to evaluate the antibiotic susceptibility among 282 gram negative anaerobic isolates recovered from clinical samples (2017-2023). Isolates were grouped as follows: *B. fragilis* (*Bf*) (100), *Bf* group not *Bf* (94), *Prevotella* spp. (22), *Veillonella* spp. (18), Others (11), *Fusobacterium* spp. (10), *Parabacteroides* spp. (10), *Porphyromonas* spp. (10) and *Bacteroides* spp. (7). Minimum Inhibitory Concentration methodology and resistance rates we based on CLSI guidelines. β-lactamase activity was determined using nitrocefin discs. *Bf* isolates were subjected to Blue Carba (BC) method. Amplification of bla_{CfiA} was performed in BC-positive *Bf*. Purified amplicons were sequenced and analysed using BLDB database.

Among the antibiotics tested, rare Ertapenem, Imipenem and Metronidazole (MTZ) resistance was encountered. Average resistance rates to Ampicillin/sulbactam (AMS) were 18% (0%-60%), Cefoxitin (FOX) 16% (0%-46%), Clindamycin 37% (0%-80%), Penicillin 51% (20%-94%) and Piperacillin/Tazobactam (PTZ) 30% (0%-72%) depending on the analysed group. Particularly noteworthy were the resistance rates of *Veillonella* spp. to PTZ (72%) and *Parabacteroides* spp. to AMS (60%). β-lactamase production was found in 45% of *Prevotella* spp., 10% of *Porphyromonas* spp. and 10% of *Fusobacterium* spp. bla_{CfiA} was confirmed in 4/6 BC-positive *Bf*, corresponding to bla_{CfiA-4} in 3/4 and $bla_{CfiA-27}$ in 1/4.

Based on our findings, β-lactam/β-lactamase inhibitor combinations, Carbapenems, FOX and MTZ are the most effective antimicrobials. Surveillance studies, such as the one we conducted, serve a dual purpose. Firstly, they enable the identification of specific groups exhibiting resistance. Furthermore, these studies provide valuable insights into the local epidemiological landscape.

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Wednesday, July 10 — One Health

1015-1115 Session IX: One Health: Anaerobes in Animals & Man

*—Indicates Presenter

THE EPIDEMIOLOGY OF *CLOSTRIDIOIDES DIFFICILE* IN A ONE-HEALTH CONTEXT

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Clostridioides difficile is an important spore-forming human pathogen associated with a serious enteric disease and represents a serious health concern to both humans and animals. The complexity of the bacterial-host relationship, which influences pathogenesis and disease progression, presents a constant challenge for epidemiological studies, control strategies and prevention planning. *C. difficile* infection (CDI) is mainly caused by strains that produce two important toxins, TcdA (enterotoxic) and TcdB (cytotoxic), during pathogenesis. *C. difficile* transferase (CDT; or binary toxin) is a third toxin produced by some *C. difficile* strains, including the epidemic PCR ribotypes 027 strain. New strains have also been reported to cause CDI with poor health outcomes, including ribotypes, 017, 014/020, 106, and 078/126. These strains differ in their source of isolation, geographic distribution, genetic repertoire, virulence determinants, and antimicrobial susceptibility profiles, which can affect their ability to cause disease and respond to treatment. For the past 20 years, CDI was considered a hospital acquired disease (HA-CDI) and affecting mainly an elderly population, especially under antibiotics use, presenting comorbidities and with previous hospitalization. However, now community acquired CDI (CA-CDI) is becoming increasingly common and is associated with adults under the age of 50, no evident risk factors and that have not been exposed to antibiotics. Although never proved, it has been postulated that there is a link between CA-CDIs and *C. difficile* from animals, food products, and the environment, since its spore form can persist in a variety of natural ecosystems, including abiotic ones. Despite increased epidemiological surveillance of *C. difficile* in developed countries, it remains difficult to accurately estimate the burden and international epidemiological trends given the lack of concerted global effort for surveillance, especially in low and middle-income countries. The One Health is an emerging concept that connects the health of humans to the health of animals and their common surroundings, and used by some countries for disease surveillance and control. In fact, it represents a suitable paradigm for understanding the emergence of CDI transmission, which is definitely still a challenge.

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EFFECTS OF COMMONLY USED MEDICATIONS ON THE GROWTH OF COMMENSAL GUT ANAEROBES AND PATHOBIONTS ASSOCIATED WITH DOMESTIC CATS AND DOGS

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The use of pharmaceutical drugs in veterinary medicine is indispensable for treating various health issues in companion animals. However, these medications, while targeting specific pathogens or conditions, often have collateral effects on the gut microbiome, particularly on anaerobic bacteria. Understanding these effects can help practitioners to make more informed decisions about medication and supplement use.

In this study, we tested the activity of 18 of the most prescribed oral medications in veterinary medicine on commensal anaerobes and pathobionts. These include a range of drug classes: antibiotics, antiparasitics, anti-inflammatory drugs, antiemetics, antihistamines, antidepressants, and antacids. We used a microdilution method to evaluate the impact of these drugs on bacterial isolates associated with the core healthy microbiome of companion animals as well as several key pathobiont species.

The results of these *in vitro* assays indicate that both antibiotics and non-antibiotic drugs can affect the growth of these organisms. Notably, these medications have been found to inhibit key anaerobic bacteria that are associated with gut health. Furthermore, specific antibiotics and antiparasitic drugs were observed to aggravate the balance of gut flora by promoting the growth of pathobionts such as *Escherichia coli* and *Streptococcus* spp., species that have been found to be elevated in pets with gut and skin health issues.

The unintended effects of commonly used drugs on the gut microbiome may promote the development of dysbiosis in the host, which is characterized by the depletion of beneficial anaerobes and a concurrent increase in pathobionts. This study highlights the need for a more strategic approach to prescribing medications in veterinary medicine, and advocates for the development of microbiome-conscious medication strategies and regulatory measures to optimize the health and well-being of companion animals. There is a pressing need for the development in clinical microbiology of standards to assess inhibition of beneficial anaerobes to quantify the impact of current and new veterinary drugs on these organisms. In addition, more studies at a synthetic community level and *in vivo* are needed to confirm these findings.

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THE MICROBIOME AND RESISTOME OF GOLDEN JACKALS IN ISRAEL

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Monitoring antimicrobial resistance (AMR) across One Health is essential, particularly in wildlife where knowledge is limited. Prior research using qPCR revealed that Israeli Golden Jackals (GJs), a synanthropic scavenger experiencing rapid population growth in the Middle East, have a high burden of extended spectrum beta lactamase (ESBL) genes. The objective of this study was to explore the microbiome-resistome context of GJs carrying ESBL genes.

Methods: Of 111 GJ fecal samples, 17 were selected for metagenomic sequencing based on DNA extract quality and presence of >2 ESBL genes. Samples were sequenced by Illumina NovaSeq6000 using the Illumina library prep protocol. The quality of raw reads was assessed using *Fastp.* Reads were assigned to species using *Kraken2.* ARGs were identified using *ARGs_OAP.* Microbiome and resistome analyses were performed in R using the *microeco* package. Procrustes analysis was performed with the *vegan package.*

Results: We identified 93 ARGs related to 16 antimicrobial classes. Tetracycline genes were the most abundant with *tet(40)* and *tet(O)* observed among all samples. Based on PCR screen, GJs had a high burden of ESBL genes: *bla*TEM (88.24%), *bla*CTX-M (47.06%), and *bla*SHV (64.71%). We used the Bray-Curtis dissimilarity index to compare β-diversity of the resistome with respect to *bla*CTX-M positivity by PCR screen. We found that the GJ resistome was significantly different with respect to *bla*CTX-M positivity (P=0.0002, Wilcoxon Rank Sum). We identified 49 bacterial families that varied widely in their relative abundance across samples. Microbiome β-diversity was also significantly different with respect to *bla*CTX-M positivity by PCR screen (P=0.0002, Wilcoxon Rank Sum). We performed Procrustes analysis to evaluate the structural correlation between the microbiome and resistome and found a moderate correlation (R=0.5187, P=0.024).

Conclusions: In this study, we show that Israeli GJs carry a broad diversity of clinically-relevant ARGs, which are associated with their microbial communities. These results indicate that shifts in the microbial community might be an important driver for ARG acquisition, or that acquisition of ARGs could drive changes in the microbiome.

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1115-1215 Session X: Anaerobes in Reproductive Health

∗—Indicates Presenter

THE IMPORTANCE OF ANAEROBIC ANTIMICROBIAL THERAPY FOR ACUTE PELVIC INFLAMMATORY DISEASE (PID)

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Purpose: To describe the microbiologic etiologies of acute PID and to illustrate the importance of including anaerobic therapy in PID treatment.

Methods and Results: Pelvic inflammatory disease (PID) is an ascending infection in the female genital tract and is a common cause of tubal-factor infertility, ectopic pregnancy, and pelvic pain. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are known PID pathogens, but in the modern era, these sexually transmitted organisms are identified in only a minority of women with PID. Bacterial vaginosis is commonly observed in women with PID, and facultative and anaerobic microorganisms associated with bacterial vaginosis are frequently identified in the upper genital tract in women with PID. A single dose of a cephalosporin plus 2 weeks of doxycycline have historically been the CDC-recommended treatment for PID, despite limited anaerobic activity of this regimen. We performed a randomized placebo-controlled trial of standard PID treatment (single dose ceftriaxone plus doxycycline x 2 weeks) with or without metronidazole 500 mg orally twice daily for 2 weeks. Clinical and microbiologic assessments were performed at enrollment and at 30 days. Among the 233 participants, persistent tenderness of the pelvic organs 1 month following therapy was less common in women receiving metronidazole than placebo (9% vs 20%, $p<0.05$). Recovery of anaerobic organisms from the upper genital tract after treatment was also less common in patients treated with metronidazole (8% vs 21%, $p<0.05$). Adherence to treatment as well as side effects were similar in each treatment arm. Following treatment, bacterial vaginosis and *Trichomonas vaginalis* infection were less common in women treated with metronidazole.

Conclusion: Anaerobic organisms are commonly involved in the microbiologic etiology of acute PID. The inclusion of metronidazole as part of the antimicrobial therapy is associated with improved clinical and microbiologic outcomes. Moreover, adding metronidazole does not affect adherence to PID treatment. Metronidazole has now become a standard component of treatment regimens for acute PID.

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APPLICATION OF ENDOLYSINS AS NOVEL ANTI-MICROBIALS AGAINST BACTERIAL VAGINOSIS

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Purpose: To investigate the antimicrobial potential of an anti-*Gardnerella* endolysin CCB7.1 against a polymicrobial bacterial vaginosis (BV) biofilm model.

Methods and results: Antimicrobial efficacy of CCB7.1 endolysin was assessed using time-kill and monospecies biofilm assays against laboratory and metronidazole resistant *Gardnerella vaginalis* isolates. A 4-species biofilm model, representative of BV dysbiosis, was also developed and optimised consisting of *G. vaginalis, Fannyhessea vaginae, Prevotella bivia* and *Mobiluncus curtisii.* Antibiofilm activity was assessed using live/dead qPCR and efficacy compared to traditional antibiotics using an *in vitro* and organotypic human vaginal epithelium model.

CCB7.1 was shown to selectively target *Gardnerella spp.,* killing all tested isolates in a time and dose dependent manner. The BV biofilm model was able to tolerate high concentrations (8×MIC) of clindamycin and metronidazole with no reduction in biofilm viability. In comparison, CCB7.1 demonstrated excellent selectivity in the biofilm model, altering the community dynamics through significantly reducing viable *G. vaginalis* within the biofilm by 50%, decreasing the composition by 1 log ($p< 0.01$). These findings were reinforced using the epithelial model, with CCB7.1 significantly reducing *G. vaginalis* tissue colonisation ($p<0.01$), with no off target cytotoxic effects on cell viability or inflammation.

Conclusions: CCB7.1 endolysin displays promising antimicrobial activity and can significantly reduce the BV bioburden compared to conventional antimicrobials such as metronidazole. Current work is ongoing, investigating novel bacterial based biotherapeutic delivery systems to administer these antimicrobial payloads.

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NOVEL *EGGERTHELLACEAE* ISOLATES FROM THE FEMALE REPRODUCTIVE TRACT

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Eggerthella-like vaginal bacteria (ELVB) have been linked with higher risk for acquiring HIV and developing pelvic inflammatory disease in women. Thirty-three Gram-variable obligately anaerobic bacteria were isolated from vaginal fluid and endometrial biopsy samples from women with bacterial vaginosis (BV) where isolates displayed ≥99.9% 16S rRNA gene sequence identity to ELVB sequences previously associated with BV. Given their clinical significance, we characterized a subset of isolates. Colonies were tiny, pinpoint, and clear on Brucella agar after 5 days at 37°C. Growth in broth culture was assessed using colony forming units as optical density could not be measured. Optimal pH for growth ranged from 5.5 to 7.5, and no growth was observed at 4.5, consistent with high pH environment in BV. ELVB were resistant to colistin and sensitive to bile, vancomycin, kanamycin, clindamycin and metronidazole. Phylogenetic analyses showed that ELVB are in the *Eggerthellaceae* family. The ELVB 16S rRNA gene had 89.7% and 89.2% sequence identities with validly named neighbors, *Cryptobacterium curtum* and *Eggerthella lenta*. Average amino acid identity (AAI) and digital DNA hybridization using genome sequences demonstrated that the ELVB were different from other validly named genera to warrant their designation as a novel genus and species. Among sequenced ELVB isolates, AAI ranged from 97.1%-100% suggesting they belong to one species despite genomic diversity. Genome sizes ranged from 1.63 Mb to 1.78 Mb. Biochemical tests showed that ELVB were asaccharolytic but were positive for several aminopeptidases indicating they can metabolize peptides. Metabolite end-product testing by NMR showed production of several bio-organic acids including pyruvate, acetate, succinate, and formate, but not lactate which was supported by pathway analyses of genomes. Availability of well characterized strains of ELVB provides the opportunity to examine the role of these bacteria in the pathogenesis of BV and other conditions.

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0830-0930 Session XI: *Advances in Public Health/Epidemiology*

∗—Indicates Presenter

GENOMIC EPIDEMIOLOGY-PROVIDING INSIGHTS INTO TRANSMISSION AND SPREAD OF PATHOGENS AT MULTIPLE SPATIAL AND TEMPORAL SCALES

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Due to the high prevalence of hospital-onset infections, interventions targeting *Clostridioides difficile* has primarily focused on disrupting transmission in healthcare settings. Here I present genomic epidemiology studies at different scales to examine evidence for the spread of *C. difficile* in healthcare settings. In the first study we performed comparative genomic analysis of prominent *C. difficile* strains RT027/ST1 and RT014/ST2, collected from large academic healthcare centers in Michigan, Houston and New York. Analysis demonstrated stark differences in the two strains. RT027/ST1 showed strong geographic clustering, with pairs of isolates within 5 single nucleotide variants being exclusively detected from the same sampling site. In contrast, RT014/ST2 showed weak geographic clustering, with closely related isolate pairs being commonly detected between geographic sites. These signatures are consistent with RT027/ST1 spreading locally within healthcare settings, with infrequent long-range spread, with RT014/ST2 showing limited evidence of spread within healthcare settings, and frequent long-range transmission via currently unknown reservoirs or vectors. In the second study we focused on a single hospital unit, sampled at extremely high resolution to comprehensively track transmission of all *C. difficile* strains. All patients entering a single medical intensive care unit (MICU) at Rush University Medical Center (RUMC) underwent daily rectal screening for *C. difficile* carriage. Overall prevalence of carriage was high, with 9.3% of patients having toxigenic *C. difficile* detected in at least one sample. Despite this high colonization pressure, among those patients not harboring *C. difficile* on admission, only 1% acquired *C. difficile* during their stay in the MICU. While admission-colonized patients appeared to present limited risk to others via cross-transmission, they themselves were at 24-times increased risk for developing *C. difficile* infections (CDI) during their hospitalization, as compared to patients not colonized on admission. These results indicate that infection prevention measures implemented at RUMC (e.g. single rooms, daily cleaning with sporicidal agents) were effective in preventing the spread of *C. difficile*. Together, these two studies support a changing paradigm for CDI, where the decrease in prevalence of the healthcare-associated epidemic strain RT027/ST1 means that to further reduce CDI rates will require identifying reservoirs and transmission pathways outside of hospitals, as well as developing strategies to prevent asymptomatically colonized patients from transitioning to active CDI.

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FUSOBACTERIUM NUCLEATUM: A MODEL PERIODONTAL PATHOGEN IN INTRAUTERINE INFECTIONS

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Intrauterine infection plays a pivotal role in adverse pregnancy outcomes including preterm birth, stillbirth, neonatal sepsis, as well as pregnancy-associated hypertension and diabetes. Development of culture-independent microbial detection technologies have greatly advanced our knowledge of microbes implicated in intrauterine infection. Increasing evidence demonstrates the involvement of oral bacteria, especially gram negative oral anaerobe *Fusobacterium nucleatum*, in adverse pregnancy outcomes. The prevalence of *F. nucleatum* in amniotic fluid and cord blood and its mechanism in intrauterine infection will be discussed.

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IDENTIFICATION OF CRYPTIC *CLOSTRIDIOIDES SPP.* AMONG *C. DIFFICILE* INFECTION (CDI) SURVEILLANCE ISOLATES COLLECTED THROUGH THE EMERGING INFECTIONS PROGRAM (EIP), 2018-2020

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Although cryptic clades of *Clostridioides difficile* were first described in 2012, recent evidence based on nucleotide-level comparisons suggests that these clades represent distinct species. It is unclear how common cryptic strains are in CDI specimens as these strains may coexist with *C. difficile* and escape detection using common diagnostic methods (nucleic acid amplification [NAA] and toxin enzyme immunoassay [EIA]). We analyzed whole-genome sequencing (WGS) data to determine the frequency of cryptic *Clostridioides* strains among isolates sequenced through EIP.

CDI cases from 10 EIP sites were identified based on positive NAA or EIA clinical results. A convenience sample of isolates from reported CDI cases in 2018-2020 that were recovered on solid selective media (cycloserine cefoxitin fructose agar) underwent WGS. Bioinformatic analyses were performed to assess genome assembly quality, determine average nucleotide identity (ANI), and identify toxin genes.

Seven out of 3,222 sequenced isolates were identified as novel *Clostridioides spp*. based on ANI values. One isolate was highly similar to sp. ES-W-0016-02 (99% ANI; Clade-III), while the other six were most similar to sp. ES-S-0054-01 (92% ANI; Clade-IV). One clinical sample was EIA+ but the recovered isolate did not carry toxin genes as determined by WGS; another clinical sample was EIA- and the recovered isolate harbored a tcdB variant. The remaining four samples were positive by NAA, but no toxin genes were detected by WGS.

We report the identification of seven cryptic *Clostridioides* strains among EIP isolates from the United States. The use of WGS enabled detection of these strains, whereas other laboratory methods would have failed to differentiate them. Future efforts are needed to determine whether cryptic strains contribute to infection and how often they are present in clinical specimens – either alone or in coexistence with *C. difficile.*

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Thursday, July 11 Host Responses in Anaerobic Infections

0930-1030 Session XII: Host Responses in Anaerobic Infections

*—Indicates Presenter

DECIPHERING HOST INFLAMMATION IN *CLOSTRIDIOIDES DIFFICILE* INFECTION: UNRAVELING THE EFFECTS OF OLFM4 IN DISEASE SEVERITY

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Although intensity of host inflammation is a better predictor of *Clostridioides difficile* infection (CDI) outcomes than pathogen burden, most current CDI therapies are pathogen-directed. While targeting *C. difficile* does control the bacterium, due to concurrent impact of antibiotics on gut microbiota, there is an increased risk of recurrent CDI. Thus, novel host-targeted, microbiota-sparing therapies are needed for CDI treatment. Neutrophils are dominant cells in the innate immune response to CDI, and intensity of neutrophil-mediated inflammation is a key driver of CDI outcomes. Utilizing intestinal epithelial cell (IEC)-neutrophil co-cultures and a pre-clinical animal model of CDI, we show that activated neutrophils exacerbate *C. difficile*-induced IEC injury. Unbiased single-cell RNAseq followed by pseudotime trajectory analysis, pathway enrichment analysis, and module scoring of gene signatures identified various neutrophil subtypes in bone marrow, blood and colonic tissue. CDI expands various neutrophil clusters that exhibit gene signatures associated with tissue damage, and our data suggest that TNF signaling could be a crucial driver for development of these inflammatory neutrophil populations. Additionally, we identified a pathogenic neutrophil population marked by Olfactomedin-4 (*Olfm4*) expression. During acute CDI, OLFM4+ cells aggregate to areas of damaged epithelium, and their numbers correlate with IEC damage score. *In vitro*, OLFM4+ neutrophils exacerbate the toxin-mediated IEC damage, and in mice, CDI increases the number of OLFM4+ neutrophils and circulating OLFM4 protein. Similarly, patients with CDI have higher amounts of circulating OLFM4 in blood compared to non-CDI controls. Finally, OLFM4-/- mice had faster resolution of diarrhea and better survival, compared to WT mice, despite similar pathogen burden. In sum, we have created the first transcriptomics atlas of CDI-induced neutrophils and identified novel neutrophil populations with pathogenic potential. Our studies also suggest that OLFM4+ neutrophils are a possible target for developing new host-directed CDI therapies.

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LOSS OF S100B ATTENUATES SEVERITY OF *CLOSTRIDIOIDES DIFFICILE* INFECTION IN MICE AND PROTECTS ENTERIC GLIA

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S100 calcium binding protein B (S100B), which is produced by enteric glia (a component of the enteric nervous system) and other cells, is increased in CDI in humans and mice. Here, we investigated whether S100B determined disease severity in the mouse model of CDI and its function on enteric glia cell death. WT and S100B KO mice (6 months old) were infected with 10^5 CFU of *C. difficile*, monitored daily (body weight, clinical score, diarrhea score, *C. difficile* shedding in stools), euthanized on day 3 post-infection (pi) and cecum tissue were processed to histopathology and TUNEL immunostaining (cell death marker). *Bifidobacterium*, *Enterobacteriacea* and *Firmicutes* in stools were measured by qPCR. *In vitro*, WT and *S100B*KO enteric glia were exposed to *C. difficile* toxins (TcdA and TcdB) and cell death was assessed by real-time apoptosis assays (Phosphatidylserine:annexin-V binding and activity of caspases 3/7). *In vivo*, levels of cecum S100B correlated positively with clinical score on day 3pi. No statistical difference in *C. difficile* shedding, as well as in *Bifidobacterium*, *Enterobacteriacea* and *Firmicutes* levels in stools were found between *S100B*-KO and WT mice during infection. However, infected *S100B*KO mice exhibited lower body weight loss (-5%), and decreased diarrhea severity (exhibiting mild or no diarrhea), resulting in lower clinical score (0-1) compared to infected WT mice (body weight loss of -15% and severe diarrhea, resulting in higher clinical score) on day 3 pi. ($p<0.001$). S100B deletion also decreased tissue damage in cecum ($p=0.0001$) and colon ($p=0.02$), as well as decreased cell death and IL-6 synthesis in the cecum compared to infected WT mice. *In vitro*, deletion of S100B on enteric glia decreased the cell death and activity of caspase 3/7 induced by *C. difficile* toxins ($p<0.001$). Our findings showed that S100B plays a crucial role in the pathogeneses of CDI, promoting severe disease and intestinal damage, including enteric glia death, independent of *C. difficile* burden and microbiota.

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TOXIN-MEDIATED ALTERATIONS IN HOST PHYSIOLOGY IMPACT *CLOSTRIDIOIDES DIFFICILE* COLONIZATION AND PATHOGENESIS

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Clostridioides difficile infection (CDI) is the leading cause of hospital-acquired diarrhea and pseudomembranous colitis, but how the toxins TcdA and TcdB reshape the physiological function of the colon to cause diarrhea and to access essential nutrients remains incompletely understood. Here, we investigated changes in solute transport and intestinal barrier function during acute CDI and recovery. Mice were infected with *C. difficile*, and we assessed intestinal permeability and solute transport at two days post-infection – or acute infection. Functional analyses revealed that intestinal permeability is increased through a dynamic tight junction pathway. Using physiological techniques, we identified a significant loss of function of Na^+-Glucose Co-transporter 1 (SGLT1) accompanied by an increased fecal glucose concentration. Furthermore, SGLT1 and two other transporters, Na^+/H^+ Exchanger 3 (NHE3) and Downregulated in Adenoma (DRA), were downregulated at the mRNA level during acute CDI. Unexpectedly, mRNA expression of SGLT1, NHE3, and DRA did not return to homeostatic levels once mice had recovered from CDI. Additionally, fecal glucose levels remained increased during recovery. Using a mutant *C. difficile* R20291 strain, we determined that the lasting changes in solute transport were dependent on the glucosyltransferase activity of the toxins. Our preliminary data might suggest that lasting changes in host solute transport may be due to toxin-mediated effects on proliferative cell populations, altering cell fate in the epithelium. Our results indicate that the function of *C. difficile* toxins alters host physiological function after acute injury, providing a sustained source of nutrients for *C. difficile* to continue to colonize the colon.

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Thursday, July 11 — Anaerobes in the Environment

1045-1145 Session XIII: Anaerobes in the Environment (Novel Anaerobes)

∗—Indicates Presenter

BARCODED OVEREXPRESSION LIBRARIES TO STUDY GENE FUNCTIONS IN GUT BACTEROIDALES

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A mechanistic understanding of how the gut microbiota influences human health is hindered by poorly annotated bacterial genomes. Functional genomic approaches can rapidly connect genes to phenotypes and accelerate the discovery of novel gene functions. However, most gain-of-function screens have been performed in *Escherichia coli*, which is phylogenetically distant from bacteria abundant in the gut. Furthermore, such screens typically require deep sequencing or strain isolation to identify beneficial genes, which is not cost-effective and laborious. To address these challenges, we developed a workflow termed Boba-seq (Barcoded Overexpression BActerial shotgun library sequencing) to build DNA barcoded overexpression libraries for competitive fitness assays using a prevalent human gut anaerobe, *Bacteroides thetaiotaomicron*, as the expression strain. Random DNA barcoding allows us to perform functional screens in a high-throughput and quantitative manner. From barcode sequencing data, we can quantify strain abundances to identify conserved genomic regions that are beneficial for growth under each condition. To demonstrate the power of this approach, we assayed genes from diverse gut Bacteroidales overexpressed in *B. theta* across hundreds of experiments. Overall, we uncovered new functions and phenotypes for 29 genes involved in carbohydrate metabolism or tolerance to antibiotics or bile salts. Gene hits encode a diverse group of proteins that consists of enzymes, transporters, regulators, and hypothetical proteins. Highlights include the discovery of a D-glucosamine kinase, a raffinose transporter, and several routes that increase tolerance to bile salts through lipid biosynthesis. Notably, Boba-seq can be readily scaled to additional assay types and can be applied to study gene functions in other bacteria.

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EXPLORING THE ROLE OF SODIUM MEMBRANE ENERGETICS IN *BACTEROIDES FRAGILIS* BILE ACID RESISTANCE

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As an opportunistic pathogen in the human gut, *Bacteroides fragilis* must adapt to bile acids, which modulate bacterial growth in part by membrane disruption. These disruptions impact the ion motive force used to generate ATP by ATP synthases (ATPase) and maintained using cardiolipin by reducing membrane permeability. While the role of hydrogen (H^+) gradients is well characterized, much remains to be understood regarding how *B. fragilis* uses alternative ion gradients, such as sodium (Na^+) under bile acid stress, and how it modulates membrane composition to minimize leakiness.

Through a combination of barcoded transposon- and RNA sequencing, we identified genes for two cardiolipin synthases and a conserved Na^+ ATPase complex important for *B. fragilis*' stress response to the microbially-modified bile acid deoxycholate. We made clean gene deletions (Δ*clsA*, Δ*clsB*, and Δ*vtpK*) and characterized their impact on *B. fragilis* growth under deoxycholate, Na^+ and H^+ stress, and intracellular ion levels through inductively coupled plasma mass spectrometry (ICP-MS).

Using *in vitro* growth curves, *B. fragilis* Δ*clsA* and Δ*clsB* strains each had longer lag phases and decreased max optical density under deoxycholate stress as well as high Na^+ and H^+. Δ*clsA* and Δ*clsB* strains also had marked losses of intracellular Na^+. The Δ*vtpK* strain was less fit under deoxycholate stress but not under other osmotic stress. *vtpK* deletion did not affect intracellular Na^+, suggesting that other enzymes help maintain Na^+ homeostasis in its absence.

Further work will characterize how the Na^+ ATPase harnesses membrane ion gradients to generate or consume ATP, particularly whether the ATPase functions primarily as a Na^+-exporting pump or as an ATP generating synthase. I will also compare the effect of membrane cardiolipin levels on ion homeostasis by using ICP-MS and targeted lipidomics. These findings suggest the importance of these widely conserved enzymes in *Bacteroides* fitness in the gut and will inform future efforts to treat *B. fragilis* infections.

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THE ROLE OF BILE ACID CONJUGATION IN GUT INFLAMMATION

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Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract that includes two subtypes Crohn's disease (CD) and ulcerative colitis (UC). The disease affects approximately 7 million people globally, but the exact etiology remains unclear. Clinical studies have shown that bile acid (BA) metabolism is altered in IBD, with higher amounts of conjugated BAs compared to microbially-modified secondary BAs, but the impact of these changes on disease progression is not well understood. Here, we employed a novel knockout mouse model with an exon deletion in the *Baat* gene, resulting in reduced host-BA conjugation, to gain insights into the role of conjugated BAs in DSS-induced colonic inflammation. *Baat* knockout (KO) mice did worse than wildtype mice, with greater weight loss, shorter colons, and enlarged spleens, indicating that the presence of conjugated BAs may be important for resistance against severe GI inflammation. We then supplemented the KO mice with a diet containing 0.3% taurocholic acid (TCA) to better understand its contributions to GI inflammation. The results showed that TCA exhibited some protective effects as evidenced by the significantly increased colon length and decreased spleen weight. However, histopathology and measurements of proinflammatory cytokines expression, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) had an upward trend, indicated that the TCA benefit may not be due to reduced inflammatory signaling. In fact, both KO and WT mice fed TCA had elevated levels of the proinflammatory BA deoxycholate (DCA) in their guts and serum, possibly contributing to increased inflammatory signaling due to bacterial metabolism of TCA. 16S rRNA gene sequencing of cecal and fecal samples showed that TCA treatment increased bacterial diversity and enriched bacteria within the *Lachnospiraceae,* which are known to metabolize TCA to DCA. In summary, conjugated BAs can improve GI pathology in the DSS-inflammation model, but this is not necessarily due to a decrease in GI inflammation. TCA and other conjugated BAs should be explored further for their roles in IBD and possible use as therapeutics.

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Thursday, July 11 *Clostridioides difficile II:* Basic Research

1315-1415 Session XIV: *Clostridioides difficile II*: Basic Research

∗—Indicates Presenter

NOVEL CELL WALL CROSSLINKING ENZYMES IN *CLOSTRIDIOIDES DIFFICILE* PRODUCE ESSENTIAL 3-3 CROSSLINKS

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C. difficile has an unusual peptidoglycan cell wall with mostly (~75%) 3-3 crosslinks rather than 4-3 crosslinks that predominate (~90%) in most bacteria. The purpose of this study was to determine whether 3-3 crosslinking is essential for viability in *C. difficile*. All previously known 3-3 crosslinking enzymes are L,D-transpeptidases with a YkuD catalytic domain. *C. difficile* has 3 YkuD-type Ldts. We used CRISPR mutagenesis to delete the three *ldt* genes alone and in combination. Surprisingly, even a triple deletion mutant was healthy and retained normal amounts of 3-3 peptidoglycan crosslinking, implying there must be at least one non-YkuD Ldt in *C. difficile*. Using bioinformatics, we noted that PG_binding_4 domains are often found in YkuD-type Ldts. *C. difficile* encodes three proteins with a PG_binding_4 domain: Ldt1 and two uncharacterized proteins with VanW domains. VanW domains are so-called because they were discovered in vancomycin resistance gene clusters in some *Enterococcus* clinical isolates, but their function is unknown. We purified soluble forms of the two *C. difficile* VanW domain proteins and demonstrated they catalyze 3-3 crosslinking *in vitro* using disaccharide tetrapeptide substrates and a fluorescent tetrapeptide substrate analog. Hence, we refer to these proteins as Ldt4 and Ldt5. It was not possible to construct a *C. difficile* mutant deleted of all five *ldt* genes, but we could build strains in which four *ldt* genes are deleted and expression of the fifth can be suppressed using CRISPRi or P_{tet}. Upon depletion of the final Ldt, *C. difficile* lost viability, rod morphology and 3-3 crosslinking. We conclude that 3-3 crosslinking is indeed essential for viability in *C. difficile* and that bacteria contain a previously unrecognized family of L,D-transpeptidases whose hallmark is a VanW catalytic domain. Database searches indicate VanW domains are common in Firmicutes but rarely found outside this phylum.

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SIDEROPHORE UTILIZATION IN *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile (*Cd*) is the leading cause of antibiotic associated diarrhea. During colonization, *Cd* must obtain essential nutrients for growth, including iron, which is used both by host cells and bacteria for many cell processes. Very little free iron is available in a mammalian host due to many iron storage mechanisms. Bacterial pathogens have evolved numerous mechanisms for acquiring iron, including small, high-affinity molecules called siderophores. Here, we first demonstrate that *Cd* can utilize a variety of siderophores as a sole iron source supporting growth. Accordingly, two putative siderophore specific ABC transporters (FhuDBGC and YclNOPQ) are induced in iron depleted conditions. To assess their role in siderophore utilization, we made the *fhuDBGC* gene deletion in *Cd*630. This mutant was unable to utilize ferrichome efficiently in iron depleted media (IDM), while complemented *fhuDBGC* restores ferrichrome utilization. It is unclear how *Cd* imports other siderophores and if YclNOPQ is involved. While many bacteria utilize siderophores produced by neighboring bacteria (xenosiderophores), some bacteria gain a competitive advantage by producing a local pool of siderophore. Although *Cd*630 does not encode siderophore biosynthesis genes, a bioinformatics analysis identified siderophore biosynthesis genes in approximately 5% of *Cd* strains. We hypothesize the ability to produce siderophore may correlate with disease severity and/or enhanced colonization. We knocked out these siderophore biosynthesis genes in strain VPI 10463. Compared to WT, the Δ*irp2* (biosynthesis impaired) strain reduces the rate that Chrome Azurol S (CAS) dye changes color, suggesting siderophore production is altered. We are currently knocking out siderophore biosynthesis in combination with the siderophore transporters to examine the potential role of siderophore production and uptake in colonization. This work provides insight into the role of siderophores in iron acquisition during *Cd* infection.

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INHIBITION OF *CLOSTRIDIOIDES DIFFICILE* SPORULATION BY THE PHOSPHOTRANSFER PROTEINS PTPA AND PTPB

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The development of a dormant spore is essential for the survival of *Clostridioides difficile* outside of the host. The conserved transcription factor, Spo0A, triggers the onset of sporulation upon phosphorylation. In *C. difficile*, two membrane-bound phosphotransfer proteins, PtpA and PtpB, inhibit the accumulation of phosphorylated Spo0A and, thus, repress spore formation. Although PtpA and PtpB exhibit identical phenotypes and gene expression patterns, their functions are not redundant, as PtpA is unable to complement a *ptpB* mutant and PtpB does not complement a *ptpA* mutant. Here, we explore the molecular mechanisms by which PtpA and PtpB impact Spo0A phosphorylation to prevent *C. difficile* spore formation. As many kinases exhibit both kinase and phosphatase activity, we investigated additional conserved residues responsible for either kinase or phosphatase activity. We generated site-directed mutants of the phosphatase-associated threonine residues (PtpA-T672R and PtpB-T668R) and kinase-associated asparagine residues (PtpA-N971D and PtpB-N787D) and assessed their role in sporulation. These data revealed that PtpA functions as a phosphatase and that PtpB requires both phosphatase and kinase activities to inhibit spore formation. Using a combination of co-immunoprecipitation and two-hybrid analyses, we investigate the direct interactions between PtpA, PtpB, and Spo0A to determine whether PtpA and PtpB form heterodimers and if they directly interact with Spo0A to repress *C. difficile* spore formation.

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1430-1530 Session XV: Anaerobic Resistance & Novel Treatments

∗—Indicates Presenter

THE IMPLEMENTATION OF FDA-APPROVED MICROBIOME THERAPEUTICS: A CLINICIAN PERSPECTIVE FOR *CLOSTRIDIOIDES DIFFICILE* INFECTIONS

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The landscape of *Clostridioides difficile* therapeutics is undergoing a paradigm change with the approval of two new live microbiome therapeutics in the past two years: fecal microbiota, live-jslm and fecal microbiota spores, live-brpk. While both therapeutics have been found to be efficacious in phase 3 trials against the prevention of recurrent *Clostridioides difficile* infection compared to antibiotics alone, the approval of these therapies lead to many new considerations for the clinician. At present, there is a dearth of head-to-head trials of these therapeutics against each other or in tandem with other therapies to prevent recurrence, such as the monoclonal antibody bezlotoxumab. The clinician must consider the historical utilization of conventional fecal microbiota transplantation and the role of this therapy in this new era. The American Gastroenterology Association's 2024 Clinical Practice Guidelines include these therapies, but the order of preference of these therapeutics still remain to be described. It is important to appreciate gaps in the present data as institutions consider how best to update clinical practices to include these new therapies.

This talk will summarize data from clinical trials of these novel therapeutics and discuss the landscape of *Clostridioides difficile* management from the perspective of an infectious diseases clinician. Scientific, logistical, and clinical challenges regarding applying trials data to real populations will be discussed, as well as considerations for improvement in equitable practices as we begin more broadly prescribe these therapies.

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OMICS EVALUATION OF OMADACYCLINE, A LOW-RISK ANTIBIOTIC FOR *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Purpose: Omadacycline is an aminomethylcycline tetracycline with a low propensity to cause *C. difficile* infection (CDI) in clinical trials. This study conducted targeted metabolomics analysis to better understand the mechanism underlying this low risk.

Method and results: In this phase 1 healthy volunteer study, 16 adults were enrolled and randomized to receive a 10-day course of oral omadacycline or vancomycin, the most frequently prescribed agent for CDI treatment. Stool samples were collected at baseline, during therapy, and end of therapy. Targeted bile acid and short-chain fatty acid (SCFA) analysis was performed using liquid chromatography-tandem mass spectrometry. Primary bile acid concentrations significantly increased compared to baseline in subjects given vancomycin ($p<0.01$) whereas no significant change was observed in the omadacycline group ($p=0.65$). Secondary bile acid concentrations significantly decreased in both groups ($p<0.001$). SCFA analysis indicated a significant decrease in butyrate and acetate levels in both groups ($p<0.05$). Decreased propionate changes were statistically significant in the vancomycin group ($p<0.001$) while they were not significant in the omadacycline group ($p=0.29$). Additionally, the omadacycline group exhibited a partial restoration of propionate and acetate levels by the end of therapy.

Conclusion: Omadacycline displayed distinct metabolomic differences compared to vancomycin in healthy volunteers. These differences might elucidate the association between omadacycline and its low propensity for CDI.

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BETA-LACTAMASE AS A PREDICTOR OF PENICILLIN SUSCEPTIBILITY FOR ANAEROBIC GRAM-NEGATIVE BACILLI

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Introduction. Beta-lactamase (BL) testing is a much relied upon indicator of penicillin (PCN) activity for anaerobes. Performance of BL is recommended for anaerobes recovered from sterile sites per Clinical and Laboratory Standards Institute (CLSI). We correlated BL results with PCN susceptibility in a large population of anaerobic gram-negative bacilli (GNB).

Methods. PCN susceptibility testing was performed, by agar dilution on 6943 clinical GNB isolates over a 3-year period (2020-2022). CLSI test method and breakpoints were applied. BL testing was performed with chromogenic nitrocefin disks (Cefinase, BD) on all GNB other than members of the *Bacteroides fragilis* group which were presumed to be positive.

Results. Of 583 BL-negative (BLn) *Fusobacterium* spp, 494 (85%) were PCN susceptible (S). Similar results were seen with BLn *Dialister* spp (n=142, 88% S) and *Leptotrichia/Pseudoleptotrichia* spp (n=67, 95% S). >95% correlation between BL-positive (BLp) and PCN resistance (R) was shown for *Campylobacter ureolyticus, Fusobacterium* spp, as well as the current and former *Prevotella* genera. However, there was poor correlation between BLn and PCN results for organisms such as *Desulfovibrio* (97% of BLn were PCN R), *Sutterella wadsworthensis* (92% of BLn were PCN R) and *Bilophila* (76% of BLn were PCN R). High PCN R results were present in *Bacteroides* spp not *B. pyogenes* or *B. heparinolytica* (3495/3504, 99.7%) and other members of the *B. fragilis* group (4013/4027, 99.65%), *Desulfovibrio* spp (41/42, 98%), *Dysgonomonas* spp (16/17, 94%) and *S. wadsworthensis* (16/17, 94%).

Conclusions. Correlation between BL and PCN test results in GNBs varies by genus and species, in part due to the existence of BL resistance mechanisms not detected by the nitrocefin disk method. Negative BL results released to providers should be followed up with PCN testing for those GNBs for which correlation with PCN is low. Given the variable BL correlation with PCN results and low use of PCN for clinical therapy of anaerobic GNB infections, BL testing of anaerobic GNBs is of limited utility.

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1530-1730 Poster Session I: Animal Models

Posters will be presented in Poster Session I
Tuesday, July 9 1530-1730.

SEXUAL DIMORPHISM IN *CLOSTRIDIOIDES DIFFICILE* INFECTIONS OF RODENT MODELS

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Clostridioides difficile infection (CDI) is responsible for the majority of identifiable hospital-related antibiotic-associated diarrhea. Epidemiological studies have consistently shown that female patients are more at risk for CDI than their male counterparts. In this study, we show that female mice developed more severe CDI than males. CDI sexual dimorphism was still apparent when animals were placed under diet conditions that exacerbated CDI severity. Unlike male mice, females undergo the estrous cycle. Thus, female mice were challenged with *C. difficile* spores when they were at the estrus, metestrus, diestrus, late diestrus/early proestrus, proestrus, or late proestrus/early estrus stages. Animals were scored for CDI sign severity, while continuously monitoring their estrous cycle stages. The resulting data showed a striking spike in CDI severity when animals were in proestrus the day before maximum CDI severity. In contrast, animals who were in estrus the day before sign scoring were protected from CDI. Infected animals showed significant increases in cytokine activity on day 1-post challenge with return to normal levels by day 3. We found direct linear correlations of CDI severity with several proinflammatory and anti-inflammatory cytokines (TNFα, IL-10, G-CSF, KC, RANTES), as well as with the sexual hormone estradiol. We also found inverse correlations with luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Prophylactic treatment of CDI also showed sexual dimorphism with females responding better to treatment than males. Interestingly, infection sexual dimorphism was reversed in hamsters, with male hamsters developing more severe CDI signs than females. In conclusion, we have shown that mice recreate many of the conditions of sexual dimorphism of human CDI.

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EFFECT OF DIET ON THE MURINE AND HAMSTER MODELS OF CDI

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Clostridioides difficile infection (CDI) can result from the disruption of the resident gut microbiota. Even though diet can lead to large changes in the gut microbiome, the effect of macronutrients on CDI is not understood. Using the murine CDI model, we assessed disease outcome and microbial community dynamics in mice fed four different diets. A high-fat/high-protein, Atkins-like diet lead to 100% mortality, while a high-fat/low-protein diet induced highly variable CDI outcomes. In contrast, mice fed a high-carbohydrate diet were protected from CDI, but shed *C. difficile* spores for a longer period. Upon antibiotic treatment, species of *Lachnospiraceae* and *Ruminococcaceae* decreased in abundance regardless of diet. 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 with effects that are intensified by high-fat/high-protein diets. In turn, high-carbohydrate diets might be protective, regardless of antibiotic-driven loss of *C. difficile* competitors. In contrast to mice, most hamsters fed a high-carbohydrate diet developed late-onset fulminant CDI. Hamsters fed a high-carbohydrate diet developed diet-specific microbiomes, with lower diversity, persistent *C. difficile* carriage, and delayed microbiome restoration. We speculate that prolonged high-carbohydrate-specific dysbiosis in these animals allowed *C. difficile* to persist in their gut where they could proliferate post-vancomycin treatment, leading to delayed CDI onset. Data from both the hamster and mouse models of CDI suggest that high-carbohydrate diets may promote antibiotic-associated dysbiosis and long-term *C. difficile* carriage, which may later convert to symptomatic CDI.

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A NOVEL BACTERIAL ISOLATE PROVIDES ELEMENTARY PROTECTION AGAINST *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Gut microbiota plays an integral role in the maintenance of gut health and colonization resistance against pathogens. Members of the *Firmicutes* phylum have been shown to provide protection against dysbiosis associated pathogens, such as *Clostridioides difficile*. We previously isolated from the fecal samples of a "humanized" mouse, a novel *Paraclostridium* strain (Isolate 691), a rod-shaped, gram positive obligate anaerobe which has 99.85% identity with *P. benzoelyticum* JC272, 99.77% identity with *P. bifermentans* JCM 1386, and 99.48% identity with *P. bifermentans* ATCC 638. Germ-free mice monocolonized with Isolate 691 survived *C. difficile* challenge and had minimal symptoms that resolved over time. However, the mice remained persistently colonized with *C. difficile* and had detectable toxins. The isolate exhibited antimicrobial activity against *C. difficile* VPI 10463 *in vitro*, potentially enhancing its protective role against *C. difficile*. These results demonstrate that a single *Firmicutes* species can provide phenotypic resistance against CDI, but may be insufficient to create an environment that prevents *C. difficile* germination and growth. Understanding the metabolic environment generated by this novel isolate and the mechanism responsible for phenotypic resistance to CDI should enhance our understanding of the role of gut microbiome in CDI.

Keywords: gut microbiome, characterization, *Firmicutes*, *C. difficile*

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SPOROBIOTA MEDIATES COLONIZATION RESISTANCE AGAINST *CLOSTRIDIOIDES DIFFICILE* INFECTION IN A GERM-FREE MOUSE MODEL

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Clostridioides difficile is the leading cause of antibiotic-associated nosocomial infections. Fecal microbiota transplantation (FMT) is an effective treatment for patients with recurrent *C. difficile* infection (rCDI), but the specific microbiome species and their functions contributing to its high efficacy remain poorly understood. Increasing evidence suggests that gut sporobiota may mediate resistance against *C. difficile* (CD). These sporobiota are members of the *Firmicutes* phylum, and can survive ethanol treatment. We previously identified 8 unrelated healthy human donors whose fecal microbiome were resistant to *C. difficile* infection and colonization when transferred to germ-free C57BL/6 mice. The present study aims to determine the efficacy of the ethanol-resistant fraction (i.e. sporobiota) of the 8 human microbiota in *C. difficile* colonization. Groups of otherwise identical germ-free (GF) C57BL/6 mice were orally gavaged with fecal suspensions from these 8 donors that had been pre-treated with ethanol. Three weeks post colonization, mice were challenged with *C. difficile* VPI 10463 spores. *C. difficile* growth, toxin production, and microbiome composition were analyzed. Of the 8 fecal microbiomes examined, 3 (37.5%) of the sporobiota retained resistance against C. difficile colonization when gavaged into GF mice (i.e. mice were asymptomatic, no CD burden or toxins in cecum), but 5 (62.5%) lost their colonization resistance (i.e. mice were asymptomatic but had detectable CD burden and toxins in cecum). Microbiome analysis identified specific taxa that were differentially abundant between the two phenotypes. These results suggest interindividual differences in sporobiota resistance against *C. difficile*, which may partially explain the differences in FMT efficacy in clinical settings.

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HIGH-FAT DIET RENDERS THE MICE SUSCEPTIBLE TO *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Gram positive *Clostridioides difficile* (*C. difficile*) is a leading cause of nosocomial enteric infections that can cause mild diarrhea to life threatening pseudomembranous colitis and death. Relationship between gut microbiota, immune response, and dietary factors can influence the disease outcome. In the present study, C57BL/6 mice were either fed with standard chow diet or high fat diet (HFD) (42% Kcal from fat) for 12-14 week. The mice were further administered with clindamycin for 1 day and then infected with 103 spores for VPI 10463. Mice fed on HFD exhibited lower survival rates compared to chow-fed mice. Also, the HFD fed mice represented more severe disease including higher weight loss, clinical scores, and bacterial colonization in the cecum. Further investigations are needed to elucidate the HFD induced changes in the gut microbiota and immune response of HFD fed mice, which could affect the susceptibility to *C. difficile* infection.

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SEX DIFFERENCE IN AGED MICE WITH CLOSTRIDIOIDES DIFFICILE INFECTION

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Background: *C. difficile* infection is more common and severe in aged females comparing to males. The microbiota plays an essential role in *C. difficile* infection. We aim to investigate sex difference in *C. difficile* infection in aged mice at microbiota level.

Methods: Aged (22 to 25-month-old) male and female C57BL/6 mice were treated with 0.5g/L cefoperazone for ten days to render them susceptible to *C. difficile* infection. Antibiotic-treated mice were challenged with 103 spores of *C. difficile* R20291 via oral gavage. Mice were monitored for weight loss and clinical signs of disease following challenge. The microbiota of animals before and after challenge was monitored by 16S rRNA-encoding gene sequence analysis.

Results: Aged male mice lost significantly less weight than female mice when infected with *C. difficile* R20291 ($p = 0.0027$). Whereas aged male mice recover after day 2 post infection, aged female mice did not. Microbiota analysis revealed that the structure of the microbiota is different between sexes at baseline, featured by aged male mice having higher abundance of *Allobaculum* and *Bifidobacterium* genera. We also found that aged male microbiota starts to diversify four days after antibiotic removal, with increased alpha diversity and the rebound of *Proteobacteria*, *Bacteroidetes* and *Actinobacteria phyla*. Aged female microbiota still maintains a low alpha diversity and is dominated by *Firmicutes* up to seven days after infection.

Conclusion: The higher diversified and resilient microbiota supports the quicker recovery in aged male mice following *C. difficile* infection.

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CELL WALL PROTEIN 2 PROTECTS MICE AGAINST *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Clostridioides difficile (*C. difficile*) infection (CDI) is a disease that causes inflammation by *C. difficile* produced toxin. Another important factor that impacts host infection and colonization is the protein array on the surface of *C. difficile*. Cell wall protein (Cwp) 2 was predicted to be good vaccine antigen. In this study, we use Cwp2 as vaccine antigen to protect against CDI in mice. Immunization of mice with Cwp2 induce potent IgG/A antibody responses against Cwp2. Anti-Cwp2 can protected mice against *C. difficile* infection and decrease *C. difficile* spores and toxin levels in the feces after infection. Then, we found that both anti-Cwp2 sera inhibited the binding of *C. difficile* vegetative cells to HCT8 cells. These results imply that Cwp2 protein may represent an effective vaccine candidate for the prevention from *C. difficile* infection (CDI).

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A NOVEL HUMANIZED MOUSE MODEL TO EVALUATE ANTIBIOTIC-INDUCED GUT MICROBIOME PERTURBATION

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Antibiotic perturbation of the gut microbiome is responsible for opportunistic infections, like primary and recurrent *Clostridioides difficile* infection. Animal models of oral antibiotic effects have several drawbacks, including differences in how antibiotics are administered. For example, water soluble and insoluble oral antibiotics cannot be directly compared via drinking water. Here, we developed a murine model where the microbiome is first acclimated to powdered chow before exposure to human-equivalent doses of pharmaceutical-grade, clinically used antibiotics on the diversity and composition of the gut microbiome. To increase human relevance, germ-free (GF) mice were given a human fecal transplant (i.e., humanized mice) before the dietary switch (powdered chow). Groups of humanized mice were exposed to four antibiotics used clinically or in clinical development to treat *C. difficile* infection for ten days (metronidazole, vancomycin, fidaxomicin, and ibezapolstat). Stool samples were collected before the diet change, a week later, two days after antibiotic exposure, and at the end of exposure (10 days of antibiotics). Additionally, the weights of the mice were tracked. During the initial change in food supply, mice gained weight. In addition, microbiome diversity and composition changes were quantified via 16S rRNA microbiome sequence analysis. In two experiments (i.e., two groups of humanized mice from different human donors), the dietary change significantly decreased alpha diversity (inverse Simpson's index) and increased beta diversity (Bray-Curtis dissimilarity) compared to untreated controls. Each antibiotic uniquely affected gut microbiome composition, allowing us to rank their effects from least to greatest (fidaxomicin, Ibezapolstat, vancomycin, and metronidazole). Collectively, our results support the use of dietary administration of antibiotics via powdered chow as a robust model of human microbiome perturbation, allowing for more accurate head-to-head estimates of these clinically used drugs.

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Tuesday, July 9 — Antimicrobials

1530-1730 Poster Session I: Antimicrobials

Posters will be presented in Poster Session I
Tuesday, July 9 1530-1730.

Tuesday, July 9 — Antimicrobials

1530-1730 Poster Session I: Antimicrobials

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Tuesday, July 9 1530-1730.

DEVELOPMENT OF NOVEL MULTI-EPITOPE FUSION VACCINE AGAINST *CLOSTRIDIOIDES DIFFICILE* INFECTION USING IMMUNOINFORMATIC APPROACH

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Clostridioides difficile infection (CDI) is the leading cause of pseudomembranous colitis – a nosocomial antibiotic associated diarrhea. One of the major problems with CDI is the high rates of recurrence, which are primarily caused by the persistence of *C. difficile* spores in the intestine. Alternative approaches are crucial for preventing infections, treating patients, and avoiding recurrences, beyond traditional antibiotic treatments. Recently, the FDA approved the first oral fecal microbiota drug for the prevention of CDI recurrence. However, there are no vaccines licensed against CDI. There are various proteins from *C. difficile* reported to have antigenic properties, among them two spore exosporium proteins CdeC and CdeM, have been reported to be critical for *C. difficile* spore adhesion/colonization.

This study aimed to develop a novel vaccine, named CdeCM, by employing immunoinformatics techniques to design and build a fusion of multiple epitopes. Protein sequences of CdeC and CdeM were analyzed and validated using standard bioinformatics tools for discovering antigenic and immunogenic epitopes. The predictions identified 7 cytotoxic T-cell lymphocyte (CTL), 3 helper T lymphocyte (HTL), and 6 B-cell potential epitopic regions capable of inducing immunogenic response. These epitopes were fused together with required linkers to develop the final fused vaccine CdeCM, which was modelled, refined, and validated. Molecular docking and molecular dynamics (MD) stimulation of CdeCM with Toll-like receptors (TLRs), B-cell receptor (BCR) complex and major histocompatibility complex (MHC) complexes, further assured its stability and binding ability to confer immune response. The in-silico analysis indicated the chimeric vaccine is stable and can confer a robust immune response in the host. In addition, the proteins of interest and the fusion protein were cloned and expressed to study their immune stimulation and protectiveness against CDI.

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ANTIMICROBIAL ACTIVITY OF EDIBLE FUNGUS FERMENTATE OF *ASPERGILLUS ORYZAE* AGAINST TOXIGENIC STRAINS OF *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile (*C. difficile*) infection (CDI) causes life-threatening diarrhea and colitis in nearly half a million people in the U.S. Only three antimicrobial agents, metronidazole, vancomycin and fidaxomicin are available for the treatment of CDI and new approaches are needed. In this study, we explored the potential antimicrobial activity of a fungal-based fermentate of the Generally Recognized as Safe (GRAS) fungus *Aspergillus oryzae,* grown in a proprietary food-grade medium against *C. difficile.* Our methodology involved producing and extracting *Aspergillus oryzae* fermentate using ethyl acetate, followed by reconstitution in methanol. Disc diffusion assays were conducted to evaluate the fermentate's efficacy against several toxigenic strains, including hypervirulent ribotype 027, with comparison to 5 µg vancomycin. To elucidate the mechanism of action, a time kill assay, spore germination assays, flow cytometry analysis, and a scanning electron microscope (SEM) image were performed and evaluated. The ethyl acetate extract of the fermentate demonstrated a significant antimicrobial activity ($p<0.05$) when compared to vancomycin toward 80% isolates, as measured by the zone of inhibition diameter. Ribotype 027 was the most susceptible strain to the fermentate (20.7 mm), but the least susceptible to vancomycin (16.6) mm. Our findings revealed that *A. oryzae* fermentate displayed bactericidal activity against *C. difficile* vegetative cells within 6 hours of exposure, in contrast to 24 hours required for vancomycin. However, neither vancomycin nor the fermentate exhibited activity against *C. difficile* spores. Furthermore, the fermentate caused extensive damage to the cell wall of *C. difficile* in a shorter duration compared to vancomycin. It induced significant cell depolarization and permeability in the *C. difficile* cell membrane. In conclusion, our study demonstrates the potent bactericidal activity of *A. oryzae* fermentate against toxigenic strains of *C. difficile*, primarily targeting the cell membrane. Further research is warranted to explore its efficacy *in vivo*.

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CLOSTRIDIOIDES DIFFICILE DOWN FOR THE COUNT! THE EFFECT OF KNOCKING OUT RELQ ON NUCLEOTIDE METABOLISM

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The bacterial stringent response is a transcriptional pathway that has been found to be conserved across bacterial species. This stringent response pathway is mediated by the signaling alarmone molecules (pp)pGpp. In *Clostridioides difficile* this stringent response has been linked to playing an important role in antibiotic resistance. However, the metabolic and regulatory effectors of the stringent response remain unknown. Our lab has successfully knocked out *C. diff relQ*, an alarmone synthetase from a *C. difficile* strain. Ion-exchange high-performance liquid chromatography methodology has been utilized to assess the effects of a *C. difficile ΔrelQ* knockout strain on intracellular nucleotide concentrations. *C. difficile* wild type strain and *C. difficile ΔrelQ* knockout strain were grown under various conditions of antibiotic stress and pellets were collected at different phases of the growth cycle. By inducing the stringent response through antibiotic exposure and RelQ's ability to utilize GDP and GTP as substrates during alarmone synthesis, the concentrations of both GDP and GTP were expected to be much higher in the knockout strain in comparison to the wildtype, since RelQ is not present to initiate alarmone synthesis. HPLC results showed differences between the knockout and wild type when treated with antibiotics, left untreated, and when compared between different growth phases. These results suggest that deleting *relQ* from *C. difficile* causes a shift in nucleotide metabolism, leading to physiological changes in the bacteria. These differences shine a light on the role that RelQ plays in the stringent response of *C. difficile*.

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A NOVEL MULTI-EPITOPE VACCINE AGAINST *CLOSTRIDIOIDES DIFFICILE* SURFACE PROTEINS

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Clostridioides difficile is a gram positive, spore-forming, obligatory anaerobic bacteria that causes diarrhea and colitis within infected individuals. One of the major problems with *C. difficile* infections (CDI) is the high rates of recurrences, which are mainly caused by persistent *C. difficile* colonization. Vaccination for prevention of CDI could be cost-effective over a range of *C. difficile* risk. However, there is no commercialized vaccine available. Various surface layer lipoproteins LP1 and LP2 [1], cell wall binding protein Cwp2 [2] and Cwp84 [3]; spore proteins CotA [4] were reported to be immunogenic and confer protectiveness against *C. difficile* infection. The goal of this project is to develop epitope-based vaccines targeting *C. difficile* colonization.

A multi-epitope novel vaccine was generated by integrating potential epitopes from the *C. difficile* surface proteins by immunoinformatic approach. Both T cell and B cell epitopes were screened and 12 Cytotoxic T Lymphocytes (CTL) epitopes, 6 Helper T Lymphocytes (HTL) epitopes, and 11 B-Cell epitopes were finally selected. These epitopes were linked together with appropriate linkers to develop an antigenic and immunogenic chimeric fusion vaccine. Structural modelling and refinement helped to develop a stable structure of the chimeric protein. Molecular docking of the fusion protein with immune cell receptors ensured the interaction and binding stability to elicit immune response. Further studies will be conducted *in-vivo* to establish its efficacy in the host.

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SIGNIFICANT AND DURABLE MICROBIOME COMPOSITIONAL CHANGES AND CLONAL ENGRAFTMENT IN A PHASE 3 TRIAL OF FECAL MICROBIOTA, LIVE-JSLM FOR RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Fecal microbiota, live-jslm (RBL) is the first FDA-approved, single-dose, microbiota-based live biotherapeutic used to prevent recurrent *C. difficile* infection (rCDI) after standard-of-care antibiotic therapy. Here, clonal engraftment of bacterial populations from RBL was assessed in participants from PUNCH™ CD3, a phase 3 trial that evaluated the efficacy and safety of RBL in preventing rCDI.

Stool samples from PUNCH™ CD3 participants were collected before study treatment and over time after study treatment and sequenced using deep shotgun methods. Species-level profiling and microbiome restoration at the taxonomic level were determined using metagenomic species (MGS) approaches and assignments. Similarity in clonal populations from stool samples after RBL administration vs the administered RBL doses was evaluated using variant loci comparisons. A randomly selected RBL dose was compared to samples from participants administered placebo for control. Per-species engraftment effectiveness was assessed by comparing participants in whom clonal engraftment was observed with participants who received an RBL dose with that species.

Among participants administered RBL with treatment success at 8 weeks (defined as remaining free of CDI recurrence), microbiota composition and diversity shifted toward more eubiotic ranges. A median of 10 species (range 0 to 78) clonally engrafted from RBL to participants administered RBL by 1 week after administration; RBL responders had significantly greater engraftment vs non-responders ($p<0.05$). No significant clonal engraftment was observed in participants administered placebo. The number of RBL responders with engraftment rose and persisted for at least 6 months. At the taxonomic class level, Bacteroidia species had the highest median engraftment effectiveness; most Clostridia species had lower engraftment effectiveness.

Durable clonal engraftment of species from RBL to participants who received RBL is associated with clinical response and underpins microbiome restoration.

Disclosures
KB, TW, and JC: Employees of Rebiotix Inc., a Ferring Company
GL: Employee of Clinical Microbiomics A/S
PM: Employee of Clinical Microbiomics A/S
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EPIMERIZATION OF OMADACYCLINE IN THE HUMAN GASTROINTESTINAL TRACT

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Background: Omadacycline is a new-generation, tetracycline-class antibiotic and has a broad spectrum of activity against bacterial pathogens including gastrointestinal pathogens such as *C. difficile*. Like tetracycline-class compounds, omadacycline undergoes epimerization that transforms native omadacycline to its C4 epimer. Omadacycline C4 epimer was previously documented in human feces, but has yet to be systematically evaluated.

Objectives: This study aimed to (i) quantify epimerization of omadacycline in human feces using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) (ii) to explore its association with host factors.

Methods: A total of 82 fecal samples were collected from 8 unique healthy volunteers in an IRB-approved phase 1 clinical trial. Native omadacycline and its C4 epimer in feces were extracted with methanol-water-ethylenediaminetetraacetic acid (ETDA) solvent containing deuterated omadacycline as internal standard. As control to evaluate the baseline-level epimerization of omadacycline, the omadacycline-free feces were spiked with omadacycline standard followed by extraction. The proportion (or relative abundance) of omadacycline C4 epimer in total omadacycline in feces or control was quantified using validated LC-MS/MS.

Results: The relative abundance of omadacycline C4 epimer in clinical trial samples ranged from 16% to 56.5% with an average of 37.4%, it was statistically significantly higher than that (~9%) in fecal blank control. Additionally, the relative abundance of omadacycline C4 epimer significantly varied among different subject samples. Fecal samples from Subject 5 contained the highest proportion value of omadacycline C4 epimer (50.0%), whereas samples from Subject 6 contained the lowest value (26.0%). The relative abundance of omadacycline C4 epimer did not differ between samples from females or males, different ages, or different days.

Conclusion: For the first time, this study systematically quantified the degree of epimerization of omadacycline in human feces and revealed significant variation in epimerization between subjects regardless of sex, age, or sampling day. This has important biological and clinical implications for future studies of omadacycline and other tetracycline-class antibiotics for use in gastrointestinal infections.

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EXPLORING NOVEL CHEMICAL ENTITIES TARGETING CD2068 TO COMBAT ANTIBIOTIC RESISTANCE IN *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Clostridioides difficile infection (CDI) poses a critical global health threat, leading to numerous annual fatalities. Given the bacterium's antibiotic resistance, there's a pressing need for effective treatment strategies. CD2068, an ATP-binding cassette transporter, has been highlighted as a pivotal target for combating antibiotic resistance in *C. difficile*. Leveraging structure-based screening, we identified Thioridazine, an FDA-approved non-antibiotic drug, as a potent inhibitor of CD2068. Thioridazine demonstrated promising binding interactions with CD2068, validated through differential scanning fluorimetry and isothermal titration calorimetry, exhibiting a moderate binding affinity. Functional assays confirmed Thioridazine's inhibitory effect on CD2068, significantly suppressing ATP hydrolysis activity by over 80%. *In vitro* studies revealed Thioridazine's additive effect in combination with standard antibiotics, effectively halting *C. difficile* growth, even at sub-lethal concentrations of conventional drugs. Moreover, at higher concentrations, Thioridazine displayed remarkable efficacy in inhibiting *C. difficile* growth, demonstrating a rapid-killing profile. Further assessments in an *in vivo* CDI mouse model showcased Thioridazine's potential in rescuing infected mice, despite a lower survival rate compared to the standard drug. Notably, Thioridazine's treatment demonstrated a significant restoration of bacterial diversity and relative gut microbiota abundance, akin to non-treated mice controls. In conclusion, Thioridazine's compelling *in vitro* and *in vivo* efficacy suggests its promise as an alternative pump inhibitor to enhance current drug treatments or as a potential clinical choice for addressing antibiotic resistance in CDI.

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INHIBITION OF *FUSOBACTERIUM NECROPHORUM* SUB-SPECIES *NECROPHORUM* AND *FUNDULIFORME* BY THE PLANT TERPENES: D-LIMONENE AND A-PINENE

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The objective of this study was the determination of antimicrobial properties of whole *Cannabis sativa* L. (hemp) crude oil extract and/or its constituents against *Fusobacterium necrophorum* subsp. *necrophorum* and *funduliforme* (strains CT59, RT38 and CT39, respectively). The compound groups within the hemp oil extract were separated based on polarity using 2-dimensional thin-layer chromatography (TLC), bioassayed on reduced medium agar plates supplemented with tryptone peptone and inoculated with *F. necrophorum* subsp. *necrophorum* (CT59) to identify zones of activity. The inhibitory zones of crude hemp oil extract were then matched (via retention factor) to zones of inhibition on TLC plates of α-pinene and D-limonene extracted and separated using the same solvent systems. The activity of bulk hemp crude oil as well as α-pinene and D-limonene were measured via 10% (v/v) serial dilutions in liquid broth. The results indicated that the crude hemp oil was not as inhibitory as some of its constituent parts against *F. necrophorum*. These data suggested that other components within crude hemp oil extract were antagonizing the activity of D-limonene and α-pinene. Therefore, we conducted a liquid bioassay with D-limonene/α-pinene and cannabidiol (CBD) and observed a loss in activity against all *F. necrophorum* strains tested. These data demonstrate: 1. D-limonene and α-pinene are bioactive against *F. necrophorum* (CT59, RT38, and CT39), and 2. CBD is antagonistic to the activity of both terpenes.

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A NOVEL ANAEROBIC ACTIVITY INDEX TO DESCRIBE THE "ANTI-ANAEROBIC" SPECTRUM OF ANTIMICROBIALS

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Background: While crucial for treatment of anaerobic infections, antibiotics with anaerobic activity may negatively impact some patient outcomes including development of graft-versus host disease in bone marrow transplant recipients and even mortality in critically ill patients. However, we lack a validated method to assess the relative anaerobic activity of commonly used antibiotics and have poor definitions of what it means for antimicrobials to be "anti-anaerobic."

Methods: A PubMed literature search using terms "microbial sensitivity test," "anti-bacterial agents" and "bacteria, anaerobic" yielded 575 papers published between January 2013-2024. Papers with <100 isolates, non-human isolates, or without antimicrobial susceptibility testing (AST) were excluded. AST for an average of 23,525 anaerobic clinical isolates per antimicrobial were summarized from 34 publications. Based on available AST literature, anaerobes were grouped as 1) *Bacteroides fragilis*, 2) other *Bacteroides* and *Parabacteroides* species (*spp*.), 3) *Fusobacterium spp*., 4) *Prevotella spp*., 5) other gram-negative *spp*., 6) *Clostridium spp*., 7) *Finegoldia magna*, 8) *Cutibacterium spp*., 9) *Parvimonas spp*., 10) other gram-positive (GP) cocci, and 11) other GP bacilli. For a given antibiotic, scores of 0, 1, or 2 were awarded for each group of anaerobes for average susceptibilities of <10%, 11-89.9%, and >/=90%, respectively.

Results: In this Anaerobic Activity Index, metronidazole received a composite score of 19, compared with penicillin (12), clindamycin (13), piperacillin-tazobactam (20), and meropenem (22).

Conclusions: Antibiotic spectrum scores have been proposed for de-escalation and stewardship interventions, but no valid score exists. A score such as this Anaerobic Activity Index might help define the spectrum of "anti-anaerobic" antimicrobials and could be a useful tool to evaluate the impact of anaerobic coverage on clinical outcomes.

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INVESTIGATING FITNESS COSTS OF FIDAXOMICIN-RESISTANT *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile infection (CDI) accounts for almost $5 billion in health care costs per year in the United States, with a staggering rate of reoccurrence even after treatment with standard-of-care antibiotics. The narrow-spectrum macrolide fidaxomicin (FDX) is also first-line treatment for CDI. Despite FDX targeting the β-clamp of RNA polymerase (RNAP), resistance to FDX has been reported in the clinic. We aim to characterize the fitness costs associated with FDX resistance. We hypothesize that developing resistance will lead to a decrease in overall fitness and virulence. Paired isolates from patients, both before and after FDX therapy were obtained from the FDX Phase III trial hosted in Japan (NCT02179658). Multilocus sequence typing confirmed genetic relatedness. Sanger and whole genome sequencing identified mutations in RNAP. Growth rate, *C. difficile* cytotoxicity, pairwise fitness competition, and sporulation assays were used to characterize fitness defects. RNAseq was performed on paired strains FD282 (MIC=0.125µg/mL) and FD292 (MIC=128µg/mL) in early log, mid-log, and stationary phase. Differential expression was confirmed by qPCR. Each of the paired isolates had unique mutations at the target, RNAP. Strain FD77 (MIC=16µg/mL) had a Val1143Leu mutation in RpoB; strain FD292 had a Val1143Asp mutation in RpoB; and strain FD131 (MIC=64µg/mL) acquired two mutations in RpoC: Arg89Gly and Arg326Cys. Growth rate assays revealed that FDX-resistant strains had variable defects, varying with media conditions. Cytotoxicity assays indicated that both FD292 and FD131 produced less toxin compared to their paired FDX-susceptible strains. RNAseq revealed extensive remodeling of the transcriptome through differential regulation of transcriptional regulators, e.g. fur, *ccpA*, translation-related proteins (i.e. ribosomal proteins), and the central carbon metabolism. Within the >1000 differentially expressed genes, we observed downregulation of genes in the pathogenicity locus (i.e., *tcdA*, *tcdE*), supporting the phenotypic data. We observed discordant regulation of genes involved in sporulation, agreeing with the decreased sporulation previously reported for FDX-resistant strains. Overall, our findings suggest that the process of developing FDX resistance is associated with fitness costs, i.e. decreased toxicity, defects in fitness and sporulation. Future studies are warranted to determine the mechanism of these fitness costs.

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NEW ANTIMICROBIAL EVG7 PREVENTS RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION IN A MOUSE MODEL BY SPARING MEMBERS OF THE LACHNOSPIRACEAE

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Clostridioides difficile is a Gram-positive bacterium that is responsible for the most common hospital acquired infections in the United States. Standard of care antibiotics to treat primary CDI include vancomycin or fidaxomicin, however in up to 30% of patients this results in recurrent CDI (rCDI). There is an urgent need for the development of therapeutics that can target *C. difficile* without affecting other protective members of the gut microbiota. The objective of this study was to assess whether a novel glycopeptide termed EVG7 (developed by the Martin research group, Leiden), could prevent rCDI when compared to standard of care vancomycin, in a mouse model of rCDI. C57BL6J mice (n=4 per treatment) were given cefoperazone in their drinking water for 5 days and a 2 day wash out before challenge with *C. difficile* 630 spores. On day 4 post challenge, mice were given either a high (0.4 mg/ml) or low (0.04 mg/ml) dose of either vancomycin or EVG7 in their drinking water for 5 days to determine if they were able to clear primary CDI, and prevent rCDI, which occurs around day 14 post challenge. Clinical signs of disease, *C. difficile* bacterial load and toxin activity were measured in the stool of mice throughout the infection. Tissue and cecal content were collected at necropsy for 16S rRNA sequencing to define the microbiota after antibiotic cessation. We report that the low dose EVG7 treated mice cleared primary CDI at day 9 and did not show any clinical signs of disease by day 14. They had significantly less *C. difficile* bacterial load and less toxin activity in their ceca compared to the other treatment groups. The 16S rRNA sequencing analysis showed an increase in members from the Lachnospiraceae Family in cecal content of mice treated with the low dose EVG7. This data suggests that EVG7 could be a promising anti-*C. difficile* agent that does not target other members of the gut microbiota, thus allowing for quicker recovery after treatment of primary CDI and preventing recurrence.

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INVESTIGATIONS ON THE MECHANISM OF DECREASED TIGECYCLINE SUSCEPTIBILITIES AMONG *BACTEROIDES* SPECIES

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Statement of purpose: Tigecycline resistance is low for *B. fragilis* group (BFG) isolates, but the resistance mechanisms is not known. Earlier investigations could not make a link between tigecycline decreased susceptibility and the carriage of *tetX* genes. However, since tigecycline is a related compound to tetracyclines, we sought to find a role for the *tetQ* genes in tigecycline resistance.

Materials/methods: 29 *B. fragilis* group isolates were selected whose tetracycline and tigecycline MICs has been recorded by agar dilution in an earlier antibiotic susceptibility study. Tigecycline MICs were also determined by an independent gradient method (Etest). We detected the *tetQ*, *tetX,* and *tetX1* genes by RT-PCR and sequenced *tetQ* genes in some selected strains. Conjugation experiments were also done to transfer the tetracycline (TET) and tigecycline (TIG) resistance phenotypes to a susceptible host. The correlation of tetracycline and tigecycline resistance were analysed by statistical methods too.

Results: The tetracycline and tigecycline MICs from agar dilution correlated significantly ($r=0.701$, $p<0.001$). The carriage of *tetQ* and *tetX* genes were 13, 2 (n=15), 7, 0 (n=7) and 3, 0 (n=7) in the TETR-TiGR, TETR-TIGS and TETS-TIGS groups ($\chi 2$, $p=0.018$ and not applicable). Additionally, tigecycline decreased susceptibility could be transferred to a susceptible host by conjugation.

Conclusions: By our experiments, we assume that some forms of the *tetQ*-harboring conjugative transposons mediate tigecycline resistance in BFG strains, but the exact molecular background should be determined in the future.

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1530-1730 Poster Session I: Clinical

Posters will be presented in Poster Session I
Tuesday, July 9 1530-1730.

1530-1730 Poster Session I: Clinical

Posters will be presented in Poster Session I
Friday, July 29 1530-1730.

CHARACTARISTICS, RISK FACTORS, AND PREVALENCE OF *CLOSTRIDIOIDES DIFFICILE* AMONG HOSPITALIZED PATIENTS IN A TERTIARY CARE HOSPITAL

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Purpose: *Clostridioides difficile* is one of the most important nosocomial infection pathogens. It is linked with many risk factors. Unfortunately, many studies have been conducted in different countries to address the *Clostridioides difficile* infections (CDI), and no studies have been conducted in Palestine. This study aims to identify the prevalence and possible risk factors associated with CDI

Methods: This was a retrospective descriptive study conducted at the AnNajah National University Hospital (NNUH) in Palestine. Data were collected for patients diagnosed with CDI who tested positive for GDH, toxins A and B between January 2018 and April 30, 2021. In addition, patient characteristics and risk factors associated with CDI were analyzed.

Results: A total of 593 participants were included in the study; 53% had hospital-acquired CDI. There was an insignificant association between participant age and CDI risk. Most patients had mild to moderate infections. Sixty-three percent of the participants were immunocompromised. About 58.5% used an antibiotic agent two weeks before CDI, and 67% were on a proton pump inhibitor (PPI). About 61.3% of patients were treated according to IDSA 2017 guidelines, and 94% responded adequately to the treatment provided.

Conclusion: There was an increased prevalence of community-acquired CDI, with a prevalence almost equal to that of hospital-acquired. In addition, most of the participants were immunocompromised. The risk factors for CDI, such as antibiotics and PPI use, were also observed with high prevalence among positive patients. Antimicrobial stewardship and the appropriate use of acid suppressors are warranted.

Keywords: *Clostridioides difficile* infection, *Clostridioides difficile* risk factors, community-acquired CDI, hospital-acquired CDI, PPI, antibiotics

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CLOSTRIDIOIDES DIFFICILE IS ASSOCIATED WITH BIOFILMS AND EARLY-ONSET DISEASE IN COLORECTAL TUMORS

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Purpose: To characterize *Clostridioides difficile* (*C. diff*) presence and activity in a cohort of human colorectal tumor samples.

Methods & Results: We investigated the presence and activity of *C. diff* in a cohort of patients with colorectal cancer (CRC) from Malaysia, who had colon tumor and paired normal tissues collected at the time of resection surgery. 16S rRNA amplicon sequencing of the tissues identified the presence of *C. diff* in 29/110 tumors and 25/110 paired, distal margin normal samples; 12 patients were positive in both tumor and normal tissue. *C. diff* was detected in these samples at low abundance (0.005-0.31% of species reads), consistent with most prior, but limited, observations. A majority of the *C. diff* positive samples were associated with biofilms (24/29 tumor, 14/25 normal) as detected by FISH. While no significant difference was found between *C. diff* presence and tumor stage or location in the colon, the bacterium was found to be present in a higher percentage of early-onset (EO) tumors (ie, in patients age <50 years), as compared to late-onset (LO) (50% EO-CRC, 21% LO-CRC, p=0.049). Additionally, the relative abundance of family Enterobacteriaceae bacteria was found to be higher in *C. diff*-positive tumors, as compared to negative tumors (17% pos, 16% neg, p=0.044). PICRUSt2 analysis to predict metabolic function from the 16S sequencing indicated that several pathways involved in bile acid turnover and oxidative response were up-regulated in *C. diff*-positive tumors as compared to negative tumors. All *C. diff*-positive tumors, were anaerobically cultured to isolate *C. diff,* with recovery of the organism from six samples to date. We plan to analyze these cultured isolates for the presence of quantitative Toxin B gene (qPCR) and bioactivity (Vero cell assays).

Conclusions: *C. diff* was detected in ~25% of tumor samples from this large CRC cohort, mostly in samples harboring biofilms, and was over-represented in early-onset tumors. Overall, these patterns may suggest a keystone role for *C. diff* in the induction or progression of certain CRC.

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ADMISSION GUT MICROBIOTA DIFFERS IN MEDICAL INTENSIVE CARE UNIT PATIENTS WHO DEVELOP HOSPITAL-ONSET *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Background: Our recent genomic epidemiology study demonstrated that carriage of toxigenic *Clostridioides difficile* at medical intensive care unit (MICU) admission greatly increased the risk of developing hospital-onset *C. difficile* infection (CDI). In this study, we further investigated the relationship between *C. difficile* carriage, acquisition, infection, and the gut microbiota in the same cohort.

Methods: Stool or rectal swab samples were collected daily from virtually every patient during their MICU stay over 9 months. DNA was isolated for 16S rRNA gene sequence-based bacterial community analysis. Samples were screened by culture for *C. difficile*.

Results: *C. difficile* carriage was detected by culture in 48/478 (10%) admission rectal swab samples with ≥3000 16S rRNA gene sequences. Higher relative abundance of the operational taxonomic unit (OTU) that included *C. difficile* was observed in samples from which *C. difficile* was cultured (linear discriminant analysis (LDA) score: 3.5, P<0.001). Surprisingly, there was not a significant difference in the overall gut microbiota structure at admission by *C. difficile* carriage based on analysis of molecular variance (AMOVA) of beta-diversity metric θYC (Fs: 1.3, P=0.13). There was also no difference at admission in microbiota structure in patients that acquired *C. difficile* compared to those that did not. Interestingly, the overall admission gut microbiota in patients that developed hospital-onset CDI (n=7) was different from those that did not based on AMOVA of θYC (Fs: 1.6, P=0.03). Differentially abundant OTUs that were higher at admission in these patients included *Bacteroides*.

Conclusion: Differences in the gut microbiota upon MICU admission, beyond *C. difficile* carriage, are associated with the development of hospital-onset of CDI and could be used to inform infection prevention strategies.

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CLINICAL VALIDATION OF MICRORNA TARGETS OF VAGINAL ANAEROBES IN BACTERIAL VAGINOSIS

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The vaginal microbiome is key to support women's reproductive health and a protective vaginal immune environment against sexually transmitted infections (STIs). A dysbiotic vaginal microbiome, named bacterial vaginosis (BV), is marked by the reduction of *Lactobacilli sp* and the increase of anaerobes, where *Prevotella bivia* (Pb) is a BV pathobiont signature. BV is also associated with increased susceptibility to acquire several STIs, including trichomoniasis. Understanding the underlying molecular mechanisms in BV is critical to propose new therapeutic and preventive actions. Micro(mi)RNAs are post-transcriptional regulators of protein synthesis that are carried in the body secretions and peripheral circulation encapsulated in extracellular vesicles (EV). Using an *in-vitro* human vaginal epithelium colonization model, we have recently reported that Pb and *Trichomonas vaginalis* cause a shared pattern of host miRNA dysregulation, providing insights into anaerobe synergisms in the evasion of the host immune system. The present study compared the mucosal EV miRNA profiles obtained from cervicovaginal secretions of reproductive-age women with BV (n=46, Nugent score 7-10) to those with normal vaginal microbiota (n=74, Nugent score 0-3). miRNA levels were quantified by the EdgeSeq NextGen global transcriptome platform. The R package *limma* was used to select miRNAs differentially expressed (DE) (FDR<0.1) in BV. Using InteractVenn, we identified 143 DE miRNAs that overlapped with DE miRNA identified in our *in-vitro* colonization model. By using miRWalk, we identified 796 validated targets of shared upregulated miRNAs, which were used to build protein-protein interaction (PPI) networks and pathway-enrichment analysis using STRING. We further found 63 significantly enriched KEGG pathways (FDR < 0.05) predicted by miRNAs upregulated both *in vivo* and *in vitro*. These findings provide evidence of a possible causative role of vaginal anaerobes in the miRNA dysregulation observed in women with BV.

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EPIDEMIOLOGY OF *CLOSTRIDIODES DIFFICILE* INFECTION IN HOSPITALIZED PAEDIATRIC PATIENTS IN SABAH, MALAYSIA

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Background: *Clostridioides difficile*, a Gram-positive, anaerobic, spore-forming bacterium, is a causative agent of infectious diarrhoea in individuals undergoing antibiotic treatment. *Clostridioides difficile* Infection (CDI) poses a substantial global public health threat, particularly in healthcare settings. Despite reports on the prevalence of *C. difficile* infections in Malaysia and other Asian countries, limited data exists regarding the burden of this infection among hospitalized paediatric populations, making CDI in children poorly understood. Our study aimed to detect and characterize *C. difficile* in hospitalized children at a major hospital in Sabah, Malaysia.

Methodology: A total of 552 suspected stool samples were examined from paediatric patients at Sabah Women & Children Hospital in Kota Kinabalu, Sabah, collected between August 2021 and January 2024. CDI was confirmed using the C. DIFF QUIK CHEK COMPLETE commercial test kit (Alere, Techlab, USA), detecting both the glutamate dehydrogenase (GDH) antigen and toxins A & B of *C. difficile*. Data analysis utilized Microsoft Excel and SPSS version 27.0 (IBM, USA). Ethical approval was obtained from the Medical Research Ethics Committee (MREC), Ministry of Health Malaysia.

Results: Of the analysed stool samples, 241 (51%) were males and 232 (49%) were females. Among them, 132 tested positive for either *C. difficile* antigen or toxins, resulting in an overall prevalence of 23.9% (132/552). Specifically, 21.4% (118/552) were positive for the antigen, while 2.5% (14/552) tested positive for the toxins. Notably, most positive cases were colonized with non-toxigenic *C. difficile* strains.

Conclusions: Our study revealed a low incidence of toxigenic *C. difficile* in the studied paediatric population. The findings underscore the need for long-term surveillance to comprehend the epidemiology of this emerging pathogen in both hospital and community-acquired CDI among children.

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ISOLATION OF *CUTIBACTERIUM ACNES* FROM CLINICAL SPECIMENS OF PATIENTS UNDERGOING MICRODISCECTOMY AND THE EFFECT OF REDOX POTENTIAL ON THE EXPRESSION OF VIRULENCE FACTORS IN THE STRAINS

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Cutibacterium acnes is an anaerobic and aerotolerant Gram-positive bacillus. The species is one of the causes of intervertebral disc degeneration and herniated disc, having been detected in 25% of cultures of patients undergoing microdiscectomy. The main goal of this study was to determine the influence of Eh on the expression of virulence factors in strains isolated from intervertebral disc tissues and skin swabs collected at the surgical incision site of patients undergoing microdiscectomy. A Multiplex Touchdown PCR was used to typify the strains and a PCR for detecting virulence genes. The biofilm production was also evaluated comparing production in thioglycolate, oxidized and reduced media.

A total of 23 patients with chronic nonspecific low back pain were included in the study. The number of samples collected ranged from 5 to 20, totaling 265 samples. The prevalence of *C. acnes* was 34.8% (8/23). A total of 89 strains were isolated and 87 were phylotyped, with 44 belonging to phylotype IB, 14 to phylotype IA1, 14 IA2, 15 II. To evaluate biofilm production, 17 strains were selected and analyzed. Most were classified as weak producers in oxidized and reduced media, followed by moderate producers in both medias and non-producers in thioglycolate media after 72 hours of incubation. The detection of virulence genes in 17 strains demonstrated the presence of 15 hyaluronidase positive, 14 sialidase positive and 03 will be confirmed. We believe that the results obtained in this study may help to elucidate the role of this microorganism in infections, through the identification of virulence factors and, also, contribute to further research being carried out to identify which of these factors may work as possible targets for the diagnosis and treatment of infections that, until now, are often underestimated.

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DECIPHERING THE SIGNIFICANCE OF *CLOSTRIDIODES DIFFICILE* DETECTION IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Purpose: *C. difficile* has been recognized as a cause of diarrhoea in hematopoietic stem cell transplantation (HSCT) recipients for many years. No studies are available from North India in HSCT patients regarding the magnitude of problem caused by *C. difficile.*

Methods: It was a prospective longitudinal study with stool samples collected at the time of hospitalization (baseline) and every week from conditioning therapy till discharge. *C. difficile* was detected by using two step algorithm of Glutamate dehydrogenase (GDH) and toxin A & toxin B (CDTAB) detection first and then cdtB gene PCR. All stool samples were also cultured on chrome agar and colonies identified using MALDI TOF MS.

Results: 212 stool samples were collected from 58 HSCT recipients (Allogeneic = 28, Autologous =30) with median age of 30 (8-64) years. Total diarrhoeal episodes were 63 with average diarrhoeal duration of 10 days. Overall GDH, CDTAB, and *C. difficile* culture was positive in 29 (14%), 15 (7%) and 14 (7%) samples respectively. PCR was positive in 14/102 (13.7%) of stool samples as DNA could be isolated in only 102 samples. Carriage rate of *C. difficile* was 13% in patients without diarrhoea.

Conclusions: This is a first prospective longitudinal study providing the prevalence of *C. difficile* infection and carriage rate in HSCT recipients in North India. *C. difficile* was more prevalent in HSCT recipients having diarrhea as compared to those without diarrhea. It was also observed that some samples, which were GDH negative and culture negative, had tested positive by PCR. This shows that PCR is more sensitive than enzyme immunoassays for detecting *C. difficile,* but the dilemma of overdiagnosis and underdiagnosis persists in *C. difficile* treatment. This study further highlights importance of baseline testing in patients undergoing HSCT for knowledge of carriage status and better management of post HSCT diarrhea.

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10 YEARS OF ANAEROBE IDENTIFICATION AT WADSWORTH CENTER: THEN AND NOW

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Anaerobic bacteria are a common cause of infections, some of which can be serious and life-threatening. Anaerobic bacteria are fastidious making them difficult to culture and hard to isolate from infected sites. The Wadsworth Center Bacterial Diseases (WCBD) Laboratory serves as the New York State (NYS) public health diagnostic and reference laboratory. For the past 10 years the WCBD laboratory has received over 2700 anaerobic bacterial isolates for identification. The isolates received are geographically diverse within NYS and from numerous specimen sources. Over the last decade, the laboratory has utilized multiple methods for identification. Identification has evolved from solely using biochemical methods to more modern methods including Microbial Identification System (MIDI), Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) MS and 16S rRNA sequencing. MALDI-TOF MS is a rapid and accurate method for the identification of bacteria species encountered in the public health laboratory, however, it cannot be used as the only method of identification, due to the anaerobic species either missing or being underrepresented in the available databases. In conjunction with MALDI-TOF MS, the WCBD uses 16S rRNA targeted next generation sequencing for identification of anaerobic bacterial isolates received. The approximate cost for testing with this algorithm is $20 per sample. This algorithm allows for identification of all samples received whether viable or not, as well as for those that may be mixed. This combined algorithm allows for improved turn-around times compared to biochemical methods, improved cost effectiveness, reduced hands-on time, and minimal culture expertise.

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A STUDY OF 36 CASES IN WHICH *BACTEROIDES PYOGENES* WAS ISOLATED

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Background: *Bacteroides pyogenes* is a microorganism found in the oral cavity of dogs, cats, and livestock. Infections in humans are often associated with animal bites, and rarely occur after sinus or oral surgery or after insertion of implants. We report clinical characteristics of 36 cases from which *B. pyogenes* was isolated.

Materials & Methods: Cases in which *B. pyogenes* was isolated from bacterial culture specimens at our hospital during a 10-year period, from 2014 to 2023, were included in the study. Each case was reviewed for patient characteristics, type of specimen isolated, source of infection, whether recurrence occurred, drug susceptibility of the strain, and duration of antimicrobial therapy.

Results: Specimens included 20 pus, 11 bile, 4 blood, 2 urine, 1 pleural effusion, and 1 spinal fluid samples. The median age was 75.5 years (Interquartile range: 59.0 –83.0), and 50% were women. Comorbidities were present in 89% of the cases. The top five comorbidities were solid tumors (36%); hypertension (22%); and dementia, chronic kidney disease, and cholelithiasis (8% each). Cholangitis was the source of infection in 30% of the cases, followed by animal bites in 19%, sinusitis and oral abscesses in 16%, surgical site infections in 8%, and other sources in 27%. Four patients (11% of the total) had recurrences, three of which were head and neck infections. Recurrent cases required approximately 1-2 months of antimicrobial therapy. Regarding drug susceptibility, all strains were susceptible to penicillins, cephamycins, carbapenems, moxifloxacin, and metronidazole; however, 38% were resistant to clindamycin.

Conclusions: Traditionally, *B. pyogenes* infections are considered to be most commonly transmitted through animal bites; however, in our study, these infections were observed to be most commonly transmitted via cholangitis. The high incidence of infections in cancer patients and high incidence of recurrence when the head and neck is infected are clinically significant.

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UTILIZING ANAEROBIC BACTERIA TO MITIGATE LUNG FIBROSIS POST-HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Commensal anaerobes, primarily found in the large intestine, also inhabit the oxygen-rich environments of the lungs. The specific roles these microorganisms play in maintaining lung homeostasis remain unclear. Hematopoietic stem cell transplantation (HCT), a treatment for various disorders, alters the lung microbiome, increasing the risk of post-transplant infections and lung fibrosis, often exacerbated by herpesvirus reactivation. Our prior work in a syngeneic-HCT murine model links a significant decline in *Lactobacillus* species in HCT mice to severe pulmonary fibrosis post-murine gammaherpesvirus 68 (MHV-68) infection.

This study identifies the specific lactobacilli reduction in HCT mouse lungs as *Lactobacillus johnsonii*. Administering live or heat-killed (HK) *L. johnsonii* intranasally to HCT mice reduced lung fibrosis significantly. Notably, HK *L. johnsonii* decreased Il17a expression in CD4 T cells without affecting Th17 cell differentiation. We have previously demonstrated that IL-17A is essential in MHV-68-induced pulmonary fibrosis in HCT mice. Increased PD-L1 expression in lung dendritic cells was observed in HK *L. johnsonii*-treated HCT mice, suggesting the PD-L1/PD-1 pathway's role in modulating inflammatory responses. *In vitro* experiments showed that bone marrow-derived dendritic cells (BMDCs) treated with HK *L. johnsonii* increased their cell surface PD-L1, leading to suppressed IL-17 production in wildtype induced Th17 (iTh17) cells, but not in PD-1 knockout iTh17 cells. Furthermore, *in vivo* administration of anti-PD-1 neutralization antibodies post-MHV-68 infection in HK *L. johnsonii*-treated HCT mice lessened the supplement's efficacy in reducing lung fibrosis.

Our results indicate that HK *L. johnsonii* holds therapeutic promise in alleviating lung fibrosis in HCT recipients, primarily through modulating inflammatory responses and reducing IL-17 production via the PD-L1/PD-1 pathway.

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INCIDENCE OF *CLOSTRIDIOIDES DIFFICILE* IN THREE HOSPITALS IN RIO DE JANEIRO STATE, BRAZIL

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Clostridioides difficile is an anaerobic, Gram-positive, spore-forming rod, and the main causative agent of antibiotic-associated pseudomembranous colitis. Some strains can produce toxins, TcdA and TcdB, and rarely binary toxin (CDT). In 2011, the epidemic strain NAP1/BI of ribotype 027 caused significant morbidity and mortality in the United States, as it is a multidrug-resistant strain with a high rate of sporulation and toxin production. For the treatment of the disease, the use of metronidazole, vancomycin, and fidaxomicin is recommended, with the latter available only in the United States (US). The purpose of this work is to analyze the incidence of *Clostridioides difficile* in three hospitals in the state of Rio de Janeiro: Clementino Fraga Filho University Hospital (HUCFF), Federal Hospital of Lagoa (HFL), and Antônio Pedro University Hospital (HUAP). For this purpose, stool samples were collected from hospitalized patients in the ITU with diarrhea and/or colitis, antimicrobial use, and suspected CDI. Following isolation on selective medium (CDBA), suggestive colonies were analyzed by MALDI-TOF MS (Biotyper). The strains were characterized as it follows: toxin production, susceptibility test, detection of toxin genes, ribotyping, detection of resistance genes, and the plasmid pCD-Metro; together with the clinical-demographic data. So far, 93 samples have been analyzed: 11 from HFL, 46 from HUAP, and 36 from HUCFF. Of these total samples, four strains of *C. difficile* were identified, three from HFL and one from HUCFF that are now being characterized. It is important to note that an outbreak of CDI was detected in the ITU of HFL, where three of 11 samples collected were positive. Finally, based on these findings, identifying circulating ribotypes in hospitals in Rio de Janeiro, as well as detecting resistance genes, is essential for understanding the strain's profile and assisting healthcare professionals in prevention measures and alternatives for CDI treatment.

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Tuesday, July 9 — One Health

1530-1730 Poster Session I: One Health

Posters will be presented in Poster Session I
Tuesday, July 9 1530-1730.

PREVALENCE AND CHARACTERIZATION OF *CLOSTRIDIOIDES DIFFICILE* IN DOGS ATTENDED AT VETERINARY CLINICS IN RIO DE JANEIRO

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Clostridioides difficile is commonly associated with nosocomial infections (HA-CDI) in humans. However, the number of community-acquired infection (CA-CDI) cases has increased in the previous decade, and investigations have identified animals as a novel source of *C. difficile* transmission. This study aimed to establish the prevalence of *C. difficile* in domestic dogs attended at veterinary clinics in Rio de Janeiro. Of the 90 samples, 14 (15.5%) were positive for *C. difficile* with 85.71% (12/14) being toxigenic, and belonging to the RT106 (71.42%; 10/14) and RT014/020 (14.28%; 2/14). The relationship between the presence of *C. difficile* and diarrhea was statistically significant (p= 0.034). Half of the *C. difficile* isolates were resistant to at least one antibiotic tested: vancomycin (VAN), metronidazole (MTZ), moxifloxacin (MOX), erythromycin (ERY), and rifampicin (RIF). One strain (7.14%; 1/14) was resistant to VAN and 14.28% (2/14) of the strains were resistant to MTZ. The presence of the pCD-METRO was found only in one strain. Concerning changes in the tcdC gene sequence, two strains (22.2%; 2/9) presented the same deletion contained in the epidemic strain, NAP1/027. The percentage of dogs positive for *C. difficile* was higher when compared to our previous study. Our results can contribute for the epidemiology of CDI in dogs in our country and bring concern about the *C. difficile* strains resistance profile in domestic dogs and the possibility of these animals spreading such strains to the community.

Financial support: FAPERJ, CNPq and CAPES

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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.

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IN VITRO BIOFILM FORMATION BY THREE ANAEROBIC BACTERIAL SPECIES ASSOCIATED WITH METRITIS IN COWS

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Background: Metritis is a complex multifactorial disease caused by a polymicrobial bacterial infection. The uterine microbiota of cows with metritis undergoes divergence and dysbiosis postpartum, which is characterized by a loss of bacterial heterogeneity, and an increase in *Fusobacterium necrophorum*, *Bacteroides pyogenes*, and *Porphyromonas levii*. Moreover, in animals that have recovered from metritis, the abundance of all three bacterial species decreases, implying that the interaction between *F. necrophorum*, *B. pyogenes*, and *P. levii* is essential for the development of metritis. To identify any synergistic relationship between *F. necrophorum*, *B. pyogenes*, and *P. levii*, we investigated the growth rates and biofilm formation of these three bacteria cultured separately and together *in vitro*.

Methods: Suspensions of *F. necrophorum*, *B. pyogenes*, and *P. levii* were prepared from log-phase culture and inoculated into 96-well or 24-well culture-treated plates and incubated anaerobically at 37°C for 3 or 5 days. The biomass of formed biofilms was evaluated by crystal violet staining. For analysis of the spatial organization of *in vitro* biofilms composed of *F. necrophorum*, *B. pyogenes*, and *P. levii*, all three species were mixed 1:1:1 and grown anaerobically for 5 days at 37 °C on cover slips in 24-well plates. Biofilms were labeled using FISH with 16S rRNA-targeted oligonucleotide probes to *F. necrophorum*, *B. pyogenes*, and *P. levii* conjugated with Fam, Cy5, and Cy3 respectively, and images analyzed using confocal laser scanning microscopy (CLSM).

Results: *B. pyogenes* and *P. levii* formed single species biofilms by 3 days after inoculation when the bacterial density was greater than 0.1 (OD600). However, no obvious biofilm was formed by *F. necrophorum* under the same conditions. Consistent results were observed when three different broths were used: brain heart infusion, Wilkins Chalgren, and chopped meat (CM) broth. CLSM imaging of FISH-labeled biofilms cultured in CM for 5 days validated the previous results. Notably, the three bacterial species formed a multispecies biofilm, which was dominated by *P. levii*, with *B. pyogenes* and *F. necrophorum* randomly distributed in the biofilm matrix. The depth of these biofilms ranged from approximately 30 to 60 µm. This arrangement highlighted the cooperative interaction and spatial distribution of the 3 bacterial species in the biofilm.

Conclusion: We successfully developed a polymicrobial biofilm model for *F. necrophorum*, *B. pyogenes*, and *P. levii* that displays mutualistic or synergistic interactions. However, *F. necrophorum* didn't form an obvious biofilm under the same conditions.

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BENEFIT OF NEW ANAEROBIC TAXA IDENTIFIED FROM THE GUT MICROBIOTA OF COMPANION ANIMALS

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A growing body of research is documenting the deleterious effects of medications commonly used in both human medicine and veterinary medicine on anaerobic bacteria that may play important roles in gut function in the host. It has been suggested that the dysbiosis arising from the removal or depletion of these gut anaerobes may contribute to the rise of inflammatory diseases in people and companion animals. Anaerobes play an important role in the health of companion animals facilitating dietary fiber fermentation and leading to the production of short-chain fatty acids (SCFAs) crucial for energy and gut barrier and bile acid metabolism for fat digestion and toxicity reduction. They also synthesize certain vitamins and support the immune system, offering protection against pathogens and promoting overall well-being.

We used culturomics to isolate microbes and whole genome sequencing to characterize the microbiome of domestic cats and dogs to develop novel probiotics with key anaerobes to restore and maintain host health. We isolated over 150 bacteria including 32 among the most prevalent taxa identified from cats and dogs, 22 novel species, and 8 new genera. The genome analysis and characterization of these taxa revealed several strains with the potential of SCFAs production, inflammation modulation, and secondary bile acid metabolism with the absence of antimicrobial resistance genes or complete resistance pathways.

This study highlights the importance of the identification and categorization of gut anaerobes and their potential in maintaining the health of companion animals. Further *in vitro* studies are needed to confirm the potential of these anaerobes and the antimicrobial susceptibility observed for recommendations on the use of drugs in companion animals.

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MOLECULAR IDENTIFICATION OF *CLOSTRIDIUM* SPECIES FROM PIG GASTROINTESTINAL TRACT IN OGUN STATE, NIGERIA

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Introduction: The gastrointestinal tract of mammals, including humans, hosts a diverse community of microorganisms, particularly bacteria, which can be relevant in the context of human diseases. Given the rising global incidences of *Clostridioides* species infections, it is imperative to investigate potential sources of bacterium transmission.

Objectives: This study aimed to assess the prevalence and toxigenicity of *Clostridioides* spp. and *C. difficile* in the gastrointestinal tract of pigs located in Ogun state, Nigeria.

Methods: Surveys were conducted using questionnaires to gather information on feeding practices from handlers at piggery farms in Ogun state. Farms routinely administering antibiotics with pig feed were selected for the collection of pig fecal samples. Anaerobic bacteria isolation and tentative identification were carried out following standardized procedures. DNA extraction from samples with isolated anaerobic bacteria was performed, and Polymerase Chain Reaction (PCR) amplification of *Clostridioides* 16SrRNA, tpi, tcd A, and tcd B gene regions was conducted. Sanger sequencing was applied to the 16SrRNA PCR products for bacterial identification and genotyping.

Results: A total of 106 pig fecal samples were obtained from three piggery farms. Anaerobic bacteria were isolated from 65 samples (61.3%), tentatively identified as Clostridium species. *Clostridioides* spp. were detected in 3 (4.6%) and 18 (27.7%) samples through the amplification of 16SrRNA and tpi, respectively. While no *C. difficile* Toxin A or Toxin B was identified, three *Clostridioides* strains were identified as *C. perfringens* strain 4928STDY7387880, Uncultured Clostridia bacterium clone TaHb20 16S, and Uncultured *Clostridioides* sp. clone GKJWQY101AAXLR.

Conclusion: The absence of *C. difficile* in the sampled pigs was observed, but the presence of *C. perfringens* and uncultured *Clostridioides* spp. suggests that pigs may serve as carriers and potential zoonotic transmitters of these bacteria.

Keywords: *C. difficile*; *C. perfringens*; Pig; Uncultured *Clostridioides*; Ogun state; Nigeria.

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MOLECULAR CHARACTERISATION OF *CLOSTRIDIUM PERFRINGENS* ISOLATES FROM PATIENTS AT GROOTE SCHUUR HOSPITAL

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Clostridium perfringens is a human pathogen and zoonotic agent causing infections that range from self-limiting food poisoning to potentially lethal, tissue destructive disease. Disease is facilitated by an array of toxins and other virulence factors. South Africa lacks published data on strains responsible for human clostridial infections.

Here we describe the characterisation of 19 *C. perfringens* isolates (collected between 2017-2020, from patients with invasive clostridial disease) using PCR-based toxin typing, E-tests and whole genome sequence (WGS) analysis.

All isolates encoded the phospholipase C (*plc*) gene. Isolates (16/19, 84%) predominantly belonged to toxin type A (only encoding the alpha toxin), while the remaining 3/19 (16%) belonged to type F (harbouring genes for both the alpha and enterotoxin (*cpe*)). Susceptibility to amoxicillin/clavulanic acid and penicillin G was seen in 18/19 (95%) and 17/19 (89%) of isolates, respectively. Only 42% (8/19) of isolates were susceptible to all tested antibiotics. Mono-drug intermediate or full resistance was detected in 7 isolates, and multi-drug resistance to metronidazole (MTZ), clindamycin (CLD), and/or a beta lactam was detected in 4/19 (21%). WGS of five metronidazole resistant isolates did not reveal known resistance factors encoding MTZ resistance, but a genetic determinant for macrolide resistance (*erm*Q) was identified in one MTZ-CLD MDR isolate. WGS further showed that one isolate was related to human isolates originating from gastroenteritis cases, although this isolate lacked the known enterotoxin (cpe) gene. The genomes of the remaining four isolates clustered with isolates originating from environmental, avian, or unidentified animal sources.

This study described *C. perfringens* toxin types and genotypes responsible for invasive disease in a small set of patients from South Africa. Identification of disease-causing isolates of possible non-human origin highlights the need for One Health approaches towards potential zoonotic pathogens such as *C. perfringens*.

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EXPLORING THE UNCHARTED: CULTIVATING BACTERIA FROM *CHIROPTERAN* (BAT) MICROBIOMES AND THE IMPERATIVE FOR CONTINUED INVESTIGATIONS

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It is recognized that the microbiome plays a pivotal role in modulating health and disease processes, especially those of mammals, of which human-associated taxa have been the primary focus. Concerning non-human mammals, bats account for ~21% of mammal species, but have one of the most understudied and unique gastrointestinal systems. Uniquely, bats possess remarkably short gastrointestinal tracks (minimal transit time), leading to a much heavier reliance on host enzymes to access energy than the rest of their mammalian relatives. Most of the available data regarding the gut microbiomes of bats detail the impacts of diet or anthropogenic forces on their gut microbial communities or the detection of specific bacterial or viral pathogens. Still, there is a paucity of described organisms; the cultivation and isolation of gut-associated taxa in bats are necessary to reveal their functions, contributions to the ecosystem within the bat gut, and the broader associated microbial biodiversity within this niche. Virtually no data concerning cultivating gut-associated taxa in bats has been addressed, and these fundamental baseline data need to be acquired. This study collected bat fecal material from a guano pile inside Tumbling Creek Cave, Protem, MO, home to a summer colony of approximately 150,000 grey bats (*Myotis grisescens*. The guano samples were subjected to cultivation-based, anoxic enrichments to study the broader bat gut microbiota. 22 bacterial isolates (16 obligate anaerobes) were recovered from these guano sample enrichments. These isolates were subjected to a polyphasic bacterial characterization approach using phenotypic, genotypic (16S rRNA and whole genome sequencing), chemotaxonomic, and in-silico approaches. Of the 16 obligatory anaerobes, 14 were assigned to validly published species. The results of this work culminated in the characterization of two novel taxa, *Lacrimispora cavernae* sp. nov. and *Paraclostridium guanonis* sp. nov., are proposed.

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Tuesday, July 9 **Environmental**

1530-1730 Poster Session I: Anaerobes in the Environment

PI-39 Unveiling Novel Microbial Guardians: Exploring the Biodegradation of Sucralose and Nitrilotriacetic Acid in Our Waterways 110

*King, J.; Miller, S.; Shears, P.; Lu, N.; Tanner, R.S.; Lawson, P.A.**

Posters will be presented in Poster Session I
Tuesday, July 9 1530-1730.

UNVEILING NOVEL MICROBIAL GUARDIANS: EXPLORING THE BIODEGRADATION OF SUCRALOSE AND NITRILOTRIACETIC ACID IN OUR WATERWAYS

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Human activities have led to the widespread introduction of synthetic anthropogenic compounds into our environment, the consequences of which remain unknown. Among these, nitrilotriacetic acid (NTA) and sucralose are prominent due to their extensive use and environmental persistence in wastewater systems. NTA is used in detergents and as a chelating agent, but may be a carcinogen in humans since it has been shown to cause urinary tract and kidney cancer in animals; although sucralose is deemed safe, recent studies have linked it to health problems. This study aimed to investigate the potential microbial degradation of these recalcitrant compounds. Closed anaerobic systems were created, where an inoculum sourced from the Norman Water Reclamation Facility was incubated in an enrichment medium with either sucralose or NTA as the sole substrate source. Monthly transfers into fresh enrichments were conducted; after several months, viable cells in both samples were finally seen using phase-contrast microscopy. Subsequent plating of these microbial communities on enrichment media with the addition of noble agar, yielded eighteen monocultures. 16S rRNA and whole genome sequencing of these monocultures unveiled three novel species representing two novel genera, 9ST, NTA5T, and NTA1T. Specifically, 9ST, a Gram-negative-staining rod belonging to the family *Azonexaceae*, was the sole novel organism found in the sucralose enrichment. NTA5T, another Gram-negative-staining rod, although isolated from the nitrilotriacetic acid, is closely phylogenetically related to strain 9ST. NTA1T is a Gram-positive-staining coccobacillus and represents a novel genus phylogenetically located in the family *Actinomycetaceae*. The names proposed for 9ST, NTA5T, and NTA1T are *Purgationimicrobium sucroalosivorans* sp.nov, *Purgationimicrobium normanense* sp.nov., and *Quisquiliimicrobium sapolyticus* sp. nov., respectively.

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Tuesday, July 9 **Antimicrobial Therapeutics**

1530-1730 Poster Session I: Antimicrobial Therpeutics

Posters will be presented in Poster Session I
Tuesday, July 9 1530-1730.

IMPACT OF AN ORALLY ADMINISTERED MICROBIOME THERAPEUTIC ON STOOL FATTY ACID METABOLITES IN A PHASE 3 RANDOMIZED TRIAL (ECOSPOR III) FOR TREATMENT OF RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION (RCDI)

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Background: In a Phase 3 double-blind, randomized trial; VOWSTTM (formerly SER-109 and hereafter VOS), an orally administered microbiome therapeutic composed predominantly of *Firmicutes* spores, was superior to placebo in reducing risk of rCDI, the primary endpoint (12.4% vs. 39.8%, respectively; RR 0.32; 95% CI, 0.18-0.58; P <0.001). Compared to placebo, VOS led to higher engraftment of dose species and rapid conversion of primary to secondary bile acids, which inhibit *C. difficile* spore germination and growth. Herein, we investigated changes in fatty acids based on literature revealing the potential role of these microbe-produced metabolites in inhibiting *C. difficile* growth and restoring gut homeostasis.

Methods: Subjects with a history of ≥3 episodes of rCDI were randomly assigned to receive either placebo or VOS following standard of care antibiotic treatment. In a *post hoc* investigation, concentrations of short, medium and branched-chain fatty acids in subjects' stool samples were measured by LC-MS/MS.

Results: At baseline, the concentrations of all measured fatty acids were comparable between VOS- and placebo-treated subjects. At week 1, concentrations of the short-chain fatty acid, butyrate, and medium-chain fatty acids, valerate and hexanoate, rapidly and significantly increased in the VOS arm compared to placebo. Furthermore, concentrations of these metabolites remained significantly higher at weeks 2 and 8 in the VOS arm compared to placebo. These changes correlated with greater engraftment of VOS dose species compared to placebo through week 8.

Conclusions: Compared to placebo, VOS demonstrated a rapid and sustained increase in concentrations of butyrate, valerate, and hexanoate in parallel with VOS engraftment. These increases were associated with a reduction in rCDI events suggesting that increased fatty acid production may improve clinical outcomes. These findings, combined with our bile acid data, suggest that VOS may modulate multiple pathways critical to the rapid interruption of the *C. difficile* life cycle.

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RECOVERY OF MICROBIOME FUNCTIONAL PATHWAYS INVOLVED IN STICKLAND FERMENTATION IN PATIENTS RECEIVING VE303, A DEFINED BACTERIAL CONSORTIUM FOR *CLOSTRIDIOIDES DIFFICILE* INFECTION (CDI)

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VE303 is a rationally defined bacterial consortium designed to promote the establishment of a gut environment resistant to CDI. VE303 is manufactured from clonal cell banks and thus overcomes limitations of fecal microbiota transplantation and other donor-derived treatments for patients with recurrent CDI (rCDI). Donor-derived treatments have inherently variable composition and quality, are difficult to scale, and have resulted in transfer of harmful emerging pathogens. Here, we assessed the functional potential of the microbiomes of VE303 recipients and placebo controls to examine if VE303 promoted a gut environment antagonistic to *C. difficile*. Specifically, proline and glycine metabolic potential were examined since species with Stickland fermentation capabilities may compete with *C. difficile*.

The CONSORTIUM Study was a Phase 2 randomized, double-blind, placebo-controlled, dose-finding study in individuals at high risk of rCDI. After completing standard-of-care antibiotics for a lab-confirmed CDI episode, subjects were randomized 1:1:1 to low-dose (LD) VE303 (1.6 x 108 CFU), high-dose (HD) VE303 (8 x 108 CFU), or placebo orally once daily for 14 days. Subjects were followed for 24 weeks to monitor safety, rCDI episodes, and gut microbiota composition. Short read metagenomic sequence data from fecal samples were assembled and annotated to characterize the functional potential of the microbiota. The diversity of genes involved in glycine and proline metabolism was compared between responders and non-responders after the first week of dosing.

VE303 HD had an acceptable safety profile and significantly reduced the risk of rCDI compared with placebo. The HD also induced superior VE303 strain colonization at 14 days than LD. Several VE303 strains encode genes involved in glycine and proline metabolism, suggesting that colonization may provide nutrient competition with *C. difficile*. VE303 recipients had a significantly larger number of detected glycine and proline reductase-related genes at 7 days than placebo recipients (Wilcoxon rank sum, $p < 0.01$). VE303 colonization may promote recovery of the microbiome with organisms that can reduce glycine and proline, and thus compete with *C. difficile* for nutrients associated with spore germination and outgrowth.

This project has been supported in whole or in part with federal funds from the Department of Health and Human Services; Administration for Strategic Preparedness and Response; BARDA, under contract number 75A50120C00177.

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SIX HUMAN ISOLATES RECAPITULATE *CLOSTRIDIOIDES DIFFICILE* RESISTANCE

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The gut microbiome is critical for resistance against *Clostridioides difficile* (CD) infection. Bacteria in the *Firmicutes* phylum are depleted in patients with CD infection and their restoration is associated with recovery. We hypothesized that a limited consortium of *Firmicutes* bacteria is sufficient for CD resistance in a gnotobiotic mouse model. We screened endospore-forming bacteria from three healthy volunteers by colonizing GF mice with human fecal suspension then challenging with CD spores. Mice colonized with different microbiome exhibited differences in clinical CD resistance, ranging from susceptible moribund to asymptomatic survival. Using fecal samples from the asymptomatic humanized mice, we isolated 6 unique *Firmicutes* species, which stably colonized GF mice. We showed that the 6-isolate (6i) mice were clinically resistant to CD challenge. CD growth, toxin production, and intestinal inflammation were significantly decreased in the 6i mice compared to CD susceptible mice. In addition, CD susceptible mice could be rescued by the 6i consortium. Fecal bile acid analysis of the 6i mice showed abundant primary bile acids but a paucity of secondary bile acids, similar to that of CD susceptible mice. In contrast, amino acids essential for CD growth, including proline, cysteine, tryptophan, leucine, and isoleucine, were significantly depleted in the 6i mice. These results demonstrate that the defined 6i consortium can recapitulate clinical resistance to CD disease, but not CD colonization, likely via amino acid competition between 6i and CD, and not via secondary bile acids inhibition of CD.

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EXAMINING GENE-ENCODED FUNCTIONAL ENGRAFTMENT PATTERNS DURING FECAL MICROBIOTA TRANSPLANTATION IN MURINE MODEL OF RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Alterations in the human gut microbiota are associated with various disease states, including susceptibility to pathogens such as *Clostridioides difficile*. Fecal microbiota transplantation (FMT), transferring stool from a healthy donor to a diseased recipient, has shown success in treating recurrent *C. difficile* infection (CDI) by restoring microbial diversity and metabolites hypothesized to restrict *C. difficile*. However, how host-environmental selective pressures influence functional engraftment and how this correlates with FMT outcome remains unclear. In a prior study using a murine model of recurrent CDI, we found that despite restoring microbial diversity, healthy human feces was unable to clear *C. difficile* in specific-pathogen-free mice. In contrast, fecal material from healthy mice effectively cleared *C. difficile*, despite comparable levels of microbial recovery between mice that received any FMT type. To further investigate gene-encoded functional composition of these microbiomes, we performed metagenomic profiling of fecal and cecal samples from these mice. Mice treated with any FMT, independent of *C. difficile* clearance, differed significantly in KEGG Orthologs (KOs) from untreated mice. Linear discriminant analysis effect size (LEfSe) identified differentially abundant KOs between mice that did or did not clear *C. difficile*, as well as across different FMT types. Significantly enriched KOs ($P<0.001$) in mice that cleared *C. difficile* were associated with amino acid utilization, aligning with work from others that has demonstrated the importance of nutrient limitation in *C. difficile* susceptibility. KOs involved in the oxidative stress response, such as cytochrome c peroxidase (Ccp), were also significantly enriched in cleared mice, suggesting a role for engrafted microbes in detoxifying host inflammatory products during CDI recovery. Future analysis will include curation of metagenome assembled genomes (MAGs) to identify strain-level contribution of these functions.

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MICROBIOTA TRANSPLANT THERAPY FOR CHILDREN WITH PITT-HOPKINS SYNDROME; A RARE GENETIC NEUROLOGICAL DISORDER

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Microbiota transplant therapy to enhance gut health and advance understanding of the gut-microbiota-brain connections in children with Pitt-Hopkins syndrome.

Pitt-Hopkins Syndrome (PTHS) is a rare neurodevelopmental disorder involving a single gene mutation, and individuals exhibit major developmental delays, intellectual disability, impaired motor function, and seizures. Most people with PTHS never talk, have limited ability to walk, and are also diagnosed with autism spectrum disorder. Around 70-80% of children and adults with PTHS also suffer from chronic GI problems (constipation, abdominal pain, or worse), which suggests a possible gut-brain axis connection modulated via gut microbiome. We designed a randomized, double-blind, placebo-controlled study with Microbiota Transplant Therapy (MTT). We enrolled 6 children (range 9-15 years, 4 males, 2 females) with PTHS. The study involved 10 days of oral vancomycin, then a bowel cleanse, followed by 12 weeks of microbiota transplant and 3-6 months of follow-up post-treatment. After 12 weeks of placebo, placebo participants were switched to MTT treatment. Substantial improvement in daily stool record (Cohen's effect size of 1.3), reduction of GI pain (effect size 0.85) and improvement in PTHS symptoms (effect size 0.52) were observed, and improvements remained after treatment stopped. 16S rRNA sequencing data analyses showed that *Faecalibacterum* and *Bacteroides* relative abundance decreased after vancomycin and increased after MTT. Engraftment calculation showed ~15% of microbes engrafted including *Faecalibacterum* and *Bifidobacterium* after MTT and increased to ~20% 3 months after treatment ended compared to ~1% for placebo. Fecal butyrate significantly increased after MTT compared to Baseline. This study was the first clinical trial done in children with PTHS, and MTT showed improvements in GI and PTHS symptoms, with engraftment of beneficial gut commensals compared to placebo even after treatment stopped.

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VARIATIONS IN HEALTHY HUMAN GUT MICROBIOTAS CONFER DISTINCT PHENOTYPES OF PROTECTION AGAINST *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Clostridioides difficile infection (CDI) is a leading cause of healthcare-associated infections. Fecal microbiota transplantation (FMT) is an effective treatment for patients suffering from recurrent CDI, yet the connection between donor microbiome and FMT treatment response remains unclear. This study aims to determine the relationship between transplanted donor microbiota and CDI resistance using a humanized gnotobiotic mouse model.

Methods: Germ-free C57BL/6 mice were orally gavaged with fecal suspension from a cohort of 30 unrelated healthy human donors (n = 5 mice for each donor microbiome). After three weeks of colonization, humanized mice were challenged with *C. difficile* VPI 10463 spores (CD). Disease severity, *C. difficile* load, toxin production and 16S rRNA sequences were analyzed. Results: Humanized mice post CD challenge showed four distinct phenotypes – Resistant (asymptomatic, no CD burden or toxins in cecum), Carrier (asymptomatic but detectable CD burden and toxins in cecum), Symptomatic (moderate disease but 100% survival), and Susceptible (severe disease with 100% mortality). Compared to the Resistant phenotype, the number of amplicon sequence variants (ASVs), number of species, Shannon Diversity and Faith Phylogenetic Diversity were significantly lower in the Carrier, Symptomatic and Susceptible phenotypes. Using Differential Abundance Analysis (DAA), we identified several taxa that were depleted or enriched in different phenotypes. Compared to Resistant phenotype, Carrier phenotype exhibited depletion of 15 species of Firmicutes, 7 species of Bacteroidota, one species each of Cyanobacteria, Proteobacteria and Verrucomicrobiota and enrichment of 2 species of Firmicutes and 4 species of Bacteroidota.

Conclusion: Human fecal microbiota exhibited variable degrees of colonization resistance against *C. difficile* challenge in a humanized mouse model. Bacterial taxa identified in this study may be candidates for colonization resistance against *C. difficile.*

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Tuesday, July 9 — Oral Microbiota

1530-1730 Poster Session I: Oral Microbiota

Posters will be presented in Poster Session I
Tuesday, July 9 1530-1730.

PERIODONTAL HEALTH STATUS OF PATIENTS WITH PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disease characterized by motor and non-motor symptoms. It is the world's second most common neurodegenerative disorder, and is considered the fastest growing neurological disorder worldwide.

The deteriorated motor skills and fine hand movements impairment accompanying this disease complicate daily oral hygiene procedures. Previous studies have shown a higher prevalence of caries, gingivitis, periodontitis, and tooth loss among this population. Surprisingly, there is no study in France focusing on the oral health of Parkinsonian patients. However, knowledge of the oral health status of French persons with PD could help provide appropriate advice and improve their oral care, in order to increase their quality of life.

So, the objective of our work is to collect data on the state of oral health of patients with Parkinson disease. Moreover, we want to see if the prescription of hygiene advice would improve the periodontal state of these patients. The inclusion criteria were major patients, with Parkinson disease, able to give written informed consent, with more than 6 teeth distributed on maxilla and mandible.

The periodontal status was evaluated using the CPITN index. Plaqueand calculus are scored on a scale of 0 to 3, using the Oral Hygiene Index Simplified. Subgingival dental plaque was obtained using sterile paper points. Total Bacterial Count was, measured using quantitative Real-time PCR, included specific bacterial counts for 9 bacteria species implicated in periodontitis. 40 patients were included in this preliminary study (ClinicalTrials.gov Identifier: NCT03827551).

The results show an improvement in the state of health at the follow-up visit, with a greater proportion of healthy tissue and a reduction in periodontal pockets. All patients had bacterial counts above the pathogenicity threshold. This study highlights poor periodontal health in patients with Parkinson's disease. The plaque index showed a significant difference between inclusion and control visit, which tends to confirm perhaps a potential beneficial impact of the advice given at the inclusion visit.

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DEVELOPMENT OF A SMALL SHUTTLE PLASMID FOR STUDIES OF ORAL *VEILLONELLA*: APPRAISAL OF POTENTIAL FOR FLUORESCENCE-BASED APPLICATIONS

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Oral *Veillonella* species are among the early colonizers of clean human tooth surfaces and likely play a significant role in natural dental plaque development. We aimed to evaluate the utility of a small single-selectable-marker *Veillonella* shuttle plasmid system by evaluating its potential for use in different oral Veillonella strains and to demonstrate the use of a gene encoding an oxygen-independent fluorescent protein to generate a fluorescent *Veillonella parvula* strain. Given that tetracycline resistance is common in oral *Veillonella*, we replaced ampicillin- and tetracycline-resistance genes in a previously described shuttle plasmid (pBSJL2) with a chloramphenicol acetyltransferase gene. The resulting plasmid (pCF1135) was successfully transformed into four strains representing *V. parvula* and *V. atypica* by either natural transformation or electroporation. We then modified pCF1135 to express the Bs2 gene that encodes an oxygen-independent fluorescent protein, yielding pCF1148. *V. parvula* SKV38 transformed with pCF1148 was approximately 16 times more fluorescent than the wild-type strain in microplate-based fluorometry experiments. Fluorescence-expressing *V. parvula* could be imaged in epifluorescence microscopy (colonies, planktonic cells) and in confocal microscopy (planktonic cells, biofilms), although photobleaching effects need to be considered. In conclusion, we anticipate that this genetic system will enable enhanced studies of different oral *Veillonella* species through the introduction of genes to explore cell properties and behavior.

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THE BIOACTIVE POTENTIAL OF ORAL MICROBIAL METABOLITES

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The oral microbiome is a complex ecosystem necessitating a balance of community members to maintain its healthy state. Poor oral hygiene, a sugar-rich diet, smoking, and genetic predispositions can disrupt this balance and cause dysbiosis. The shift towards gram-negative, pathogenic species paves the way for several oral diseases, namely dental caries, gingivitis, and periodontitis.

While pathogens are able to drive disease progression through production of virulence factors (lipopolysaccharides, fimbria, and gingipain), few studies investigate the role of oral microbial secondary metabolites. Identifying bioactive metabolites within the context of gingivitis is foundational, as gingivitis is the reversible precursor to the more severe periodontitis. From a 35-day experimental gingivitis study, we correlated 320 molecular features from the human oral metabolome using mass spectrometry of saliva samples. One annotated feature, Cyclo(Val-Pro), is a diketopiperazine (DKP) hypothesized to be a quorum sensing-signal, regulating gene expression and stimulating biofilm formation. Biofilms are critical to disease, providing a haven for obligate anaerobic pathogens. Previous reports identified other DKPs, Cyclo(Leu-Pro) and Cyclo(Phe-Pro), upregulated in periodontitis. Our work aims to validate the biofilm-promoting activity of DKPs and examine the extent of their role in pathogenesis.

Beyond biofilms, we have demonstrated oral microbial extracts stimulating NF-κB signaling in macrophages. As macrophages can promote cancer proliferation through inflammation, metabolites within these extracts may have the extended ability to stimulate cancer development. We identified several organic, pre-fractionated extracts from oral microbes exhibiting cancer-proliferative activity. The overarching goal of this project is to identify, characterize, and understand the regulation of cancer, inflammation, and biofilm relevant metabolites.

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Wednesday, July 10 — Student Poster Presentations

1245-1400 Student Poster Session: Student Presentations

Posters will be presented in Student Presentation Session
Wednesday, July 10 1245-1400

MOLECULAR MECHANISMS OF MUCOSAL COLONIZATION BY *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile is a Gram-positive, spore-forming anaerobe and is the most common cause of antibiotic-associated diarrhea, representing a major public health threat. Although intestinal dysbiosis and immune deficiency are known to favor *Clostridioides difficile* infection, the underlying host-pathogen interactions that promote colonization and persistence in humans are unknown.

Recent *in vitro* and animal studies have provided compelling evidence that *C. difficile* associates with the colonic mucus layer during infection, creating interest in investigating mucus as a putative site of colonization. Using an improved quantitative model of *in vitro* adherence to mucus, we explored the molecular mechanisms underlying association with mucus. Specifically, we tested the potential for extracellular appendages, Type IV pili (T4P), and flagella to mediate mucosal adherence. Using immobilized mucins and gene-interruption mutants of the primary T4P (PilA1) and flagellar (FliC) subunits, flagella were found to be important for adhesion, while T4P were not necessary. Recent research has shown that *C. difficile* exhibits substantial phenotypic heterogeneity via phase variation through site-specific DNA recombination. Different groups have described a novel regulatory mechanism that facilitates the ON/OFF expression of co-regulated virulence factors of *C. difficile*, including flagella and type IV pili. These data suggest that the phenotypic variants generated by flagellar switch inversion have distinct capacities for adherence.

To understand the relationship between flagellation and adherence, we directly measured levels of flagellation for *C. difficile* mutants along with swimming motility and mucosal adherence. TEM data show that phase variant strains with flagellin production switched ON (*flgON*) and the *pilA1*- mutant have more flagella compared to the wild type and that the reverse *flgOff* mutant shows less flagellation. In mucin binding experiments, *flgON* shows greater binding than *flgOFF*, but both bind less compared to the wild type. Finally, our measurements of swimming motility for these strains show a binary phenotype with mutant strains showing either swimming comparable to wild type (*pilA1*-, *flgOn*) or no swimming (*flgOff*, *fliC*-). Based on these results, we hypothesize that i) direct adhesion of flagella to host mucins and ii) inverse regulation of T4P and flagella combine to modulate *C. difficile* adhesion to host mucus. Work is ongoing to identify these additional factors that contribute to mucin binding and may impact ability of *C. difficile* to colonize and cause disease.

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STRUCTURAL BASIS OF ATYPICAL ALARMONE SYNTHESIS IN *CLOSTRIDIOIDES DIFFICILE*

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Statement of Purpose: Point mutating *Clostridioides difficile* RSH and RelQ protein sequences to match homologous bacterial residues will elicit why *C. difficile* has an atypical alarmone synthesis mechanism. Another component is to isolate the *C. difficile* RelC protein and identify its true functionality as a putative synthetase enzyme to allow future research to target these three proteins for the elimination of *C. difficile* infections.

The *C. difficile* stringent response (SR) is regulated by enzymatic activity of a dual function Rel-Spo alarmone synthetase-hydrolase (RSH), a monofunctional small alarmone synthetase (RelQ), and possibly by a third putative synthetase (RelC). The alarmones created by all other Gram-positive bacteria are pentaphosphate (pppGpp), tetraphosphate (ppGpp), and triphosphate (pGpp). However, *C. difficile* exclusively produces pGpp and its mechanism of synthesis is different from all previously studied. The first objective of this project is mutational analysis of RSH and RelQ to determine the structural basis of their unusual activity. We have identified sites where these enzymes diverge from conserved synthetase sequences and will generate point mutants to see if restoring the sequences allows for synthesis of longer alarmones. DeepMind's AlphaFold will be used to predict which amino acid residues are vital for proper enzyme function that can be initially targeted by point mutagenesis. The use of predictive protein folding software will greatly minimize the time needed to identify and mutate residues crucial in unique alarmone synthesis. The other objective of this project is to determine whether RelC, despite low sequence homology to other synthetases, is a functional alarmone synthetase. Through various gene isolating techniques, RelC can be induced and incubated with γ-32P ATP and guanosine nucleotides to characterize its unknown function. The results of this project will inform future studies of atypical alarmone synthesis in *C. difficile* as possible targets for drug development and the eradication of pathogenic infections caused by *C. difficile*.

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RelQ-MEDIATED ALARMONE SIGNALING REGULATES GROWTH, SPORULATION, AND STRESS-INDUCED BIOFILM FORMATION IN *CLOSTRIDIOIDES DIFFICILE*

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Biofilm production and sporulation play a crucial role in bacterial antibiotic tolerance and novel approaches are needed to combat it. The bacterial stringent response (SR) is a conserved transcriptional reprogramming pathway mediated by the nucleotide signaling alarmones, (pp)pGpp. The SR has been implicated in antibiotic survival in *Clostridioides difficile*, a biofilm- and spore-forming pathogen that causes resilient, highly recurrent *C. difficile* infections. The role of the SR in other processes and the effectors by which it regulates *C. difficile* physiology are unknown. *C. difficile* RelQ is a clostridial alarmone synthetase. These studies delete the *C. difficile* SR gene *relQ*, revealing the pivotal role of RelQ in regulating SR-dependent phenotypes in this organism. Deletion of *relQ* dysregulates *C. difficile* growth in unstressed conditions, affects susceptibility to antibiotic and oxidative stressors, and drastically reduces biofilm formation. While wild-type *C. difficile* displays increased biofilm formation in the presence of sub-lethal stress, the ΔrelQ strain cannot upregulate biofilm production in response to stress. Deletion of *relQ* slows spore accumulation in planktonic cultures, but accelerates it in biofilms. This work establishes biofilm formation and sporulation as alarmone-mediated processes in *C. difficile* and reveals the importance of RelQ in sporulation and stress-induced biofilm regulation. This work explores innovative methods to diminish the protection against extracellular stress conferred by sporulation and biofilm production in both *Clostridioides difficile*.

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GAME OF MICROBES: UNVEILING THE ROLE OF ORAL MICROBIOME METABOLITES IN HEALTH AND DISEASE

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Purpose: Despite recent advancements in microbiome research, understanding the biological functions of metabolites within the oral microbiome remains limited. This study aims to elucidate the role of specialized oral microbe metabolites in disease development and oral health maintenance.

Methods: We conducted comprehensive biological evaluations and a metabolomics study to explore the bioactivities and profiles of oral microbiome metabolites. Saliva samples were collected from twenty participants during the induction of gingival inflammation (gingivitis). Metabolites were extracted using a combination of solid-phase extraction and liquid-liquid extraction methods optimized for saliva samples. Subsequently, the extracted metabolites underwent derivatization and were subjected to liquid chromatography-mass spectrometry (LC-MS) analysis for comprehensive profiling.

In parallel, biological evaluations were performed to assess the bioactivities of oral microbiome metabolites. Macrophage-like cells (264.7) and oral carcinoma cell lines were cultured and treated with the identified metabolites. Proliferation and differentiation assays were conducted using standard protocols, and cell viability was assessed using MTT assays.

Results: Our investigation unveiled a diverse range of bioactivities associated with oral microbiome metabolites, including two novel peptide-derived molecules capable of inducing the proliferation and differentiation of macrophage-like cells. Additionally, we identified a peptidic molecule (unpublished) from anaerobe *Veillonella parvula* exhibiting the ability to enhance oral carcinoma cell proliferation *in vitro*.

Furthermore, our metabolomics analysis identified secondary metabolites, such as cyclo(L-Tyr-L-Pro), with regulatory properties for quorum sensing and inflammatory marker secretion, indicating a specialized role for secondary metabolites in oral health maintenance. Notably, we observed a metabolic lag during dysbiosis recovery of the oral cavity, suggesting a lingering presence of signaling molecules for pathogenic microbe proliferation or total oral metabolome modification following microenvironmental stress.

Conclusion: Our findings highlight the significant role of oral microbiome metabolites in health mediation within the oral cavity and systemic disease progression. The identification of novel metabolites and their regulatory functions provides insights into oral microbiome dynamics and opens new avenues for therapeutic interventions in oral carcinoma, periodontal diseases, and related systemic ailments.

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DETERMINING THE ROLE OF CYSTEINE RICH PROTEIN CdeC IN EXOSPORIUM ASSEMBLY AND MORPHOGENESIS OF *CLOSTRIDIOIDES DIFFICILE* SPORES

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Clostridioides difficile, is an obligate anaerobe and a leading cause of nosocomial diarrhea worldwide, associated with approximately 470,000 infections annually in the United States alone. *C. difficile* infections (CDI) are treated with antibiotics, however upon cessation of treatment, there is a risk of recurrence due to the production of spores in the gut reinitiating infection. Prior work demonstrates that the outermost layer of the spore, the exosporium, interacts with host surfaces, and recent evidence suggests it plays an important role in recurrence and persistence. Consequently, disruption of exosporium layer assembly alters the progression of the disease. Our lab identified a cysteine (Cys)-rich protein, CdeC, that is essential for exosporium assembly, and its absence leads to an aberrantly assembled exosporium layer, while overexpression leads to thickening of this layer. Interestingly, CdeC can self-assemble into organized inclusion bodies (IB's), displaying lamella like layers when heterologously overexpressed in *E. coli* similar to layers seen within the spore, and seems to undergo post-translational processing. CXXC motif are present in the CdeC amino acid sequence, they have been implicated in redox sensing, disulfide bond formation, as well as metal binding. However, the functional consequences of CdeC self-processing and CXXC motifs remain unclear. Here, through construction of CdeC variants in the CXXC motifs, we explore their functional consequences in self-assembly through western blot analysis and TEM. Selected variants are used to complement a cdeC mutant strain and assess their impact in exosporium assembly. Additionally, using N-terminal Edman degradation, we identified the CdeC cleavage-site, and by site-directed mutagenesis of the neighboring residues, the impact of processing in CdeC´s function is being explored. These results will fill in the gap of the molecular basis of how CdeC drives the assembly of the exosporium layer.

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EFFECT OF BUTYRATE ON ENTERIC GLIA RESPONSE TO *CLOSTRIDIOIDES DIFFICILE* TOXINS

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Microbiota play an important role on susceptibility to *C. difficile* infection (CDI). One of the products of the microbiota is butyrate, which has been shown to be protective against CDI. In this study, we evaluated whether butyrate could modulate the response of enteric glia (a cellular component of the enteric nervous system) to *C. difficile* toxins. *In vitro,* rat enteric glia was incubated with TcdA or TcdB alone or in combination with sodium butyrate 1h prior to toxins challenge. After 18h incubation, enteric glia was collected to analyze cell death (by using a RealTime-Glo annexin and caspase 3/7 activity assays) and levels and expression of *bcl2* (an antiapoptotic factor), *S100B* and *IL-6* by qPCR. *C. difficile* toxins induced enteric glia death followed by increased levels of caspase 3/7 and downregulation of *bcl2*, as well as upregulated the expression of pro-inflammatory mediators (*S100B* and *IL-6*). In high concentration, butyrate (200 µM) potentialized the effects of *C. difficile* toxins in promoting enteric glia death, as shown by increased levels of fosfatidilserin-annexin V binding and caspase 3/7 activity ($p<0.0001$). Whereas low concentration of butyrate (0.2 µM) decreased enteric glia death and their caspase 3/7 activity ($p<0.001$) induced by *C. difficile* toxins. In addition, low concentration of butyrate (0.2 µM) by itself upregulated *bcl2* expression compared to control cells ($p<0,0001$), as well as decreased the downregulation of *bcl2* ($p<0.02$) and upregulation of IL-6 ($p<0.0002$)induced by TcdB. Further, low concentration of butyrate (0.2 µM) also diminished *S100B* upregulation induced by TcdA ($p=0.04$). Our findings suggest that low and high concentration of butyrate can differentially affect the susceptibility of enteric glia to *C. difficile* toxins, being the low concentration protective against the deleterious effects of *C. difficile* toxins. Thus, these findings brought new perspectives on how microbiote-derived products can modulate the response of enteric glia to the *C. difficile* toxins.

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MAPK MUTATIONS ALTER HOST-MICROBE INTERACTIONS AT THE COLONIC EPITHELIAL BARRIER

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Colorectal cancer (CRC) is a heterogenous disease, with many tumors carrying mutations in the mitogen-activated protein kinase (MAPK) pathway, including $Kras^{G12D}$ and $BRAF^{V600E}$. Dysbiosis of the gut microbiome has been implicated in CRC development, yet the relationship between host oncogenes and microbial dysbiosis is largely unknown. It was previously found that mice expressing the human $BRAF^{V600E}$ allele exhibited a disorganized mucosal bilayer and increased mucin production in the mid-proximal (MP) colon. Expression of $BRAF^{V600E}$ in $Apc^{Min/+}$ mice colonized by enterotoxigenic *Bacteroides fragilis* promoted MP and distal serrated colon tumors with a distinct immune microenvironment compared to the distal tubular adenomas seen in mice expressing $Kras^{G12D}$. We hypothesize that the manifestation of these distinct phenotypes in tumorigenesis is driven, at least in part, by unique interactions between the gut microbiota and specific oncogenes presented by the colonic epithelium. Herein, we explored host-mediated barrier dysfunction and the impact on microbial composition and function in specific-pathogen-free C57BL/6 (WT), $BRAF^{V600E}Lgr5^{Cre}$ (BL), and $Kras^{G12D}Lgr5^{Cre}$ (KL) mice, 7 days after antibiotic treatment as a trigger for dysbiosis. By PAS staining, BL, but not WT or KL, mice displayed disruption of the mucous bilayer structure in the MP colon. RNA sequencing of colon tissue suggested genotype and colon region-specific transcriptional changes in mucins 2, 3, and 20, a result with which we are further validating. 16S rRNA amplicon sequencing of fecal samples demonstrated significant enrichment of *Blautia* and *Akkermansia* and depletion of *Turicibacter* in BL mice relative to WT, with a trend toward enrichment of *Akkermansia* in KL mice. PICRUSt analysis revealed enrichment of glycosaminoglycan degradation and galactose metabolism pathways and reduction in fatty acid biosynthesis in BL mice. These data suggest that $BRAF^{V600E}$ promotes mucosal barrier dysfunction and a distinct pattern of microbial dysbiosis, supporting the hypothesis of specific oncogene-microbiota interactions crucial to development of the CRC tumor microenvironment.

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UNCOVERING THE MICROBIAL GUARDIANS: EXPLORING BACTERIAL SPECIES DIVERSITY PROTECTING CONVENTIONAL MICE FROM *CLOSTRIDIOIDES DIFFICILE* INFECTION

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The commensal gut microbiota plays a crucial role in safeguarding the host against enteric pathogens, including *Clostridioides difficile* (*C. difficile*), a devastating healthcare-associated infection. Mouse models have been extensively employed to investigate the gut microbiota. Since conventional mice are generally resistant to *C. difficile* infection (CDI), *C. difficile* challenge in these animals requires pre-treatment with antibiotic cocktails. Germ-free (GF) mice colonized with murine *Firmicutes* are resistant to *C. difficile* challenge. In the present study, we examined the association between *Firmicutes* diversity and richness and the degree of phenotypic resistance against *C. difficile* challenge. C57BL/6 germ-free mice were orally gavaged with increasing dilutions (1:5, 1:10, 1:20, 1:40) of murine *Firmicutes*, thereby generating groups of mice with varying degrees of microbiome diversity and richness. Mice harboring lower dilutions were asymptomatic and survived the challenge, but remained colonized with *C. difficile* with detectable toxins (i.e. carrier mice). In contrast, mice colonized with higher dilutions of *Firmicutes* succumbed to CDI (i.e. susceptible mice). *C. difficile* toxin levels at the endpoint (14 days post-infection) were significantly lower in the carrier mice compared to susceptible mice. 16S rRNA sequencing analysis identified eight taxa that were differentially abundant between the two groups. Rescue of carrier mice with murine *Firmicutes* consortium significantly reduced *C. difficile* burden and toxins, but surprisingly, failed to eliminate *C. difficile* from colonization These results demonstrate that *Firmicutes* diversity and richness correlate strongly with phenotypic *C. difficile* resistance, and suggest that an adequate number and diversity of *Firmicutes* is required for resistance against CDI.

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IN PATIENTS WITH SUSPECTED SEPSIS, EARLY ANTI-ANEROBIC ANTIBIOTIC EXPOSURE INCREASES RISK OF ACUTE KIDNEY INJURY

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Acute kidney injury is a frequent complication of sepsis, contributing to morbidity and mortality. Recent observational studies and animal modeling have revealed that depletion of gut anaerobes via anti-anaerobic antibiotics increases risk of mortality. The relationship between gut anaerobe depletion and acute kidney injury (AKI) is unknown.

Methods: We conducted a retrospective cohort study of patients admitted to intensive care units at the University of Michigan with suspected sepsis from 2015-2020. Eligible subjects were age 18-89 and had suspected sepsis based on Rhee criteria. We excluded patients transferred from outside institutions, readmissions, and those with end-stage renal disease or abnormal creatinine (>1.2mg/dL) throughout their hospitalization. We defined AKI using the Kidney Diseases Improving Global Outcomes consortium definition (>50% increase in baseline creatinine within 7 days of hospitalization). We compared AKI onset in patients, who did and did not receive early anti-anaerobic therapy, using logistic regression analysis.

Results: We identified a cohort of 12,897 patients with suspected sepsis with intravenous antibiotics administered within 24 hours of admission. Of these 10.9% (1,404) developed AKI more than 24 hours after admission and 89.1% (11,493) did not. Early exposure to anti-anerobic antibiotics was more common among patients that developed AKI than patients that did not (P<0.001). In patients treated with anti-anaerobic antibiotics, 11.9% developed AKI whereas among patients treated with anaerobe-sparing antibiotics, only 6.4% developed AKI. After adjusting for sex, severity of illness, and burden of medical comorbidities, the odds ratio of developing AKI in patients who did and did not receive anti-anaerobic therapy was 1.68 (CI 1.41-2.02, P<0.001).

Conclusion: Among patients with suspected sepsis, early exposure to anti-anaerobic antibiotics increases patients' risk of developing AKI. Future study will be necessary to determine the mechanisms by which this occurs.

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Thursday, July 11 — Diagnostic Methods

1530-1730 Poster Session II: Diagnostic Methods & Microbiology

Posters will be presented in Poster Session II
Thursday, July 11 1530-1730.

ASSESSING RIBOTYPING METHODS FOR *CLOSTRIDIOIDES DIFFICILE* STRAIN TYPING: A COMPARISON BETWEEN USA AND EUROPE

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Background: PCR-fluorescent ribotyping (RT) is a prevalent method in Europe and the USA for typing *C. difficile* strains, which requires maintenance of a comprehensive library of known isolates for comparative studies. In 2015, our laboratory developed and validated a widely adopted PCR-fluorescent ribotyping technique aligning our library with the prevailing European isolates. The purpose of this study was to assess how our US-based library has changed over time compared to the European library.

Method: *C. difficile* isolates, representative of the most prevalent European strains, were obtained from UCLouvain, Brussels, Belgium, and transported to our central laboratory. Isolates were cultured, identified as toxigenic *C. difficile* via PCR, and ribotyped. Ribotypes from our method were compared to results obtained from the Belgium lab.

Results: Fluorescence PCR ribotyping was performed on 54 European isolates from 24 distinct ribotypes including RT014 (n=6), RT020 (n=3), RT070 (n=3), RT017 (n=3), and RT506 (n=3). Of these, 68% of isolates (37 out of 54) exhibited concordance between the two laboratories. Furthermore, 29% of ribotypes (7 out of 24) consistently demonstrated concordance, with all strains identified under the same RT. USA RT014-020 (n=16) strains were identified as European RT014 (n=7), RT020 (n=3), RT709 (n=4), or RT076 (n=2) suggesting potential genetic shifts since the synchronization of our original library.

Conclusions: Moderate ribotype concordance was observed between USA- and European-*C. difficile* strains typed via PCR-fluorescence ribotyping. The maintenance of a standardized worldwide strain library is imperative for effective monitoring of this global pathogen.

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USE OF BACTERIOCINS TO IMPROVE LIVE BIOTHERAPEUTIC PRODUCT PURITY ASSAYS

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Background: Live Biotherapeutic Products (LBPs) are biological products that contain live organisms and are intended for use as a drug. LBP microbial purity testing should demonstrate that the product is free of microbial contaminants. Product organism growth can obscure the results of culture-based microbial purity methods often used. Our approach is to use bacteriocins to selectively inhibit product strain growth, thereby improving detection of contaminants.

Methods: Activities of four Class IIb bacteriocins were assessed against 20 *Lactobacillus* strains and 21 anaerobic and facultatively anaerobic bacteria using a soft agar dilution assay. A probiotic containing lyophilized Plantaricin S (PS) sensitive *L. delbrueckii* strain was suspended in phosphate buffer, serially diluted, and plated onto MRS agar supplemented with 2.5 µM PS. Colonies that grew were identified, using MALDI-TOF-MS.

Results: PS inhibited 17/20 lactobacilli with variable sensitivity. NC8 inhibited two lactobacilli strains. Lactocin 705 and Plantaricin JK did not inhibit any of the strains tested. The *L. delbrueckii* strains and an *L. jensenii* strain were inhibited by 0.5 µg/ml PS, whereas 4 other *L. jensenii* strains were sensitive to 50 µg/ml PS. Of the non-lactobacilli tested, the Gram-negative strains were not inhibited by PS. Most of the Gram-positive strains tested were inhibited by ≥500 µg/ml PS, while 50 µg/ml PS inhibited the growth of 1/5 *S. aureus*, 1/6 *L. monocytogenes*, 2/3 *E. faecalis*, and 1/2 *E. faecium* strains tested. Supplementation of MRS agar with 2.5 (5.8 µg/ml) µM PS resulted in a 7-8 log reduction of viable *L. delbrueckii* recovered from a probiotic product compared to the control and improved detection of *L. reuteri* and *L. salivarius* contaminants.

Conclusions: PS exhibited potent killing activity against some of the lactobacilli strains tested and the non-lactobacilli strains tested were typically less sensitive to PS compared to the lactobacilli tested. Media supplementation with PS significantly reduced the product viable counts and improved detection of product contaminants. These data suggest that PS is a promising candidate for LBP purity reagent development.

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TAXONOMIC ANALYSIS OF NEW PREVOTELLA SPECIES ISOLATED FROM AN EQUINE CLINICAL SPECIMEN

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Background: In recent years, as the relationship between humans and pet animals has become closer, microorganisms newly recognized as pathogenic bacteria for humans have emerged, and from the perspective of One Health, infections in animals close to humans have become more important. Moreover, it is necessary to identify the microorganisms responsible for this disease.
In this study, we performed biochemical and molecular phylogenetic analyses of a group of newly identified Prevotella species from clinical equine isolates.

Materials and Methods: In this study, we investigated five clinically isolated strains suspected of being *Prevotella* spp., isolated from horses and deposited in our laboratory. Biochemical tests were performed using Rapid ID 32A (bioMérieux). Colony shape and bacterial morphology were observed after culturing on Brucella HK agar medium (RS) (Kyokuto Pharmaceutical Industries, Ltd.) at 37°C under anaerobic conditions for 48 h. The isolated colony was applied for MALDI-TOF MS analysis and the spectra were measured. Identification analysis was performed using a MALDI Biotyper (BRUKER) with MBT Compass 4.1. In addition, bacterial fatty acid analysis and full-length 16 rRNA gene sequences of the strain were determined, and phylogenetic analyses were performed.

Results and discussion: The five horse-derived clinical isolates used in this study formed non-pigmented colonies when cultured anaerobically for 48 h on Brucella HK agar medium (RS). Mass spectrometry analysis using MALDI-TOF MS showed that all five strains had score values of 1.999 or less for any candidate species, making it difficult to identify among the bacterial groups registered in existing databases. In contrast, phylogenetic analysis of the full-length 16 rRNA gene sequences revealed that they formed a cluster independent of existing *Prevotella* species. The most closely related sequences, *Prevotella massiliensis* (AF487886) and *Prevotella phocaeensis* (LN998069), exhibited 94.94% and 91.2% homology, respectively. In biochemical tests, those five strains showed almost the same characteristic properties and distinguished them from the existing species. The results of this study showed that the five clinical isolates belonged to an independent taxonomic group and were new bacterial species belonging to the genus *Prevotella*.

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DEEP LEARNING IMAGING ANALYSIS OF CANCER-ASSOCIATED BACTERIAL METABOLIC STATES

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Gut bacteria can produce carcinogenic metabolites. For example, *Clostridium scindens* can produce the carcinogenic bile acid deoxycholic acid (DCA) from cholic acid. It is unknown whether imaging methods can allow for the identification of DCA-producing *C. scindens* states. Our main aim is to develop robust deep-learning models for bacterial classification and segmentation of light microscopy images.

Methods: We generated light microscopy images of *C. scindens* using the TissueFAX at 100X magnification. We imaged *C. scindens* co-cultured with either *Bacteroides thetaiotaomicron* (associated with low DCA production) or *Bacteroides vulgatus* (associated with high DCA production). For classification, we explore three methods: Convolutional neural network model de novo, utilizing pre-trained models: ResNet and DenseNet, and employing a dataset encompassing 33 bacterial species (DIBaS). For segmentation, we utilize nnUnet training with images of *C. scinden*s and *B. vulgatus*.

Results: For the classification of *C. scindens* cultured with cholic acid (DCA-producing cancer-associated state) or without cholic acid (control), a de novo model performed an accuracy of 0.34, F1 score of 0.39. However, pre-trained with DenseNet achieved an accuracy of 0.75 and an F1 score of 0.85, and ResNet achieved an accuracy of 0.85 and an F1 score of 0.91. ResNet with DIBaS, achieved an accuracy of 0.89 and an F1 score of 0.90. Similarly, in the classification of "*C. scindens* + *B. thetaiotaomicron*" and "*C. scindens* + *B. vulgatus*," the highest performance was observed when utilizing ResNet with DIBaS, achieving an accuracy of 0.86 and an F1 score of 0.83. Additionally, the nnU-Net segmentation model achieved a Dice coefficient of 87% for *C. scindens* and 76% for *B. vulgatus*

Conclusion: The image-based deep learning models may be able to identify carcinogen-producing cancer-associated bacterial states. Pre-trained models perform better due to their prior exposure to extensive datasets and capture patterns for improved accuracy.

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COMPARATIVE ANALYSIS OF GENOMIC RELATEDNESS WORKFLOWS FOR INVESTIGATING HEALTHCARE-ASSOCIATED *CLOSTRIDIOIDES DIFFICILE* INFECTIONS

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Clostridioides difficile is an opportunistic pathogen responsible for over half a million infections annually in the US and is a prominent cause of nosocomial infectious diarrhea. *Clostridioides difficile* infections (CDI) can have symptoms that range from mild diarrhea to severe life-threatening conditions, such as fulminant colitis. With the high incidence of CDI in healthcare and long-term care facilities, characterizing isolate relatedness and transmission networks has become essential for infection control and prevention. In the past decade, whole genome sequencing (WGS) has emerged as a vital tool for epidemiological investigations due to the increased resolution it offers for delineating transmission clusters, and the ability to characterize clinically relevant genes (virulence factors, antimicrobial resistance). Genomic relatedness workflows utilize diverse methodologies, including allele-based (core and whole genome multi-locus sequence typing; cgMLST, wgMLST), and nucleotide variation approaches (single nucleotide polymorphism, SNP; insertion-deletions, Indels), each with their unique strengths and challenges. Here, we evaluate four publicly described genomic relatedness workflows (a wgMLST, two reference-independent SNP, and a reference-dependent SNP) along with an in-house developed mutation event (ME; SNP and short Indels) workflow on a collection of 92 *C. difficile* isolates from 7 retrospective outbreak investigations across New York State. Results from genomic binning and relatedness analyses were largely consistent across workflows. Of the 92 isolates, 82 were placed in genomic bins consistent with *C. difficile* MLST profiles, while 10 isolates were singletons. Among isolates in genomic bins, 44 were grouped into 14 clusters comprised of closely related isolates. Specifically, the in-house ME and the reference-based SNP workflows clearly delineated closely related and unrelated isolates within genomic bins. Integrating a robust, reproducible, and scalable genomic relatedness workflow with WGS will enhance the ability of healthcare institutions to implement infection control measures and prevent CDI.

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AMPLICON SEQUENCE VARIANTS (ASVS) CONTAIN IMPORTANT INFORMATION ABOUT THE GUT MICROBIOME

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The microbiota of the large intestine, the gut microbiome, have numerous impacts on human health, and high-throughput techniques have allowed extensive DNA sequencing of these organisms. The overall composition of the microbiome is most frequently assessed using 16S amplicon sequencing. This technique leads to partial sequences of the 16S gene, Amplicon Sequence Variants (ASVs), that can be used to ascertain the phylogeny of the source microbes. Commonly, these ASVs are grouped by similarity (clustered) into Operational Taxonomic Units (OTUs), which are then used as the unit of analysis when interrogating the composition of the microbiome. However, the practice of clustering ASVs into OTUs can collapse ASVs with distinct behaviors into the same taxonomic unit. Here, we show that, in the context of 16S-targeted amplicons of the V4 region, ASVs are a reproducible and informative level of analysis. ASVs sequenced with overlapping primers in the V4 region have a low error rate and are found in similar proportions of people in large, geographically separated, healthy human studies. Instead of representing sequencing error or a single population with sequence variation, closely related ASVs can exhibit distinct behaviors. In particular, there exist multiple pairs of common ASVs which would cluster into the same 99% OTU, but are significantly less likely to be found in the same person. The members of these pairs also have different relationships to the rest of the ASVs in the microbiome. Elucidating the many complex relationships between microbial populations in the gut microbiome becomes more difficult when distinct populations are grouped into the same taxonomic unit. ASVs allow the disaggregation of these distinct populations, even when they have very similar regions of their 16S gene.

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BENCHMARKING A NOVEL QUANTITATIVE PCR-BASED MICROBIOME PROFILING PLATFORM AGAINST SEQUENCING-BASED METHODS

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Purpose. The primary aim of this research was to develop and validate quantitative PCR (qPCR) assays for a wide range of gut commensals and pathogens, traditionally overlooked due to inadequate genomic information. We sought to compare the efficacy of these qPCR assays against established next generation sequencing (NGS) microbiome profiling methodologies - 16S amplicon and metagenomic sequencing.

Methods. We designed 110 species-specific qPCR assays for gut Bacteria and Archaea using a novel proprietary *in silico* pipeline and validated the assays against stool samples from three healthy donors. The quantitative microbiome profiles were compared to taxonomic profiles generated by standard bioinformatic approaches for 16S amplicon and metagenomic sequencing. The qPCR assays were assessed for their ability to detect low abundance microbes and their correlation with NGS results, focusing on taxonomic resolution and limits of quantification.

Results. The qPCR assays demonstrated high concordance with advanced metagenomic and the ineffectiveness of 16S amplicon methods to achieve species-level assignments. qPCR microbiome profiles were more highly correlated with the most current bioinformatic methods than the bioinformatics methods were to each other. The profile comparisons also highlight how the continued use of older bioinformatics protocols can limit results and lead to misinterpretation of data. Notably, qPCR identified taxa undetected or underestimated by metagenomic approaches, revealing limitations in current bioinformatics tools for differentiating closely related species and quantifying low abundance taxa.

Conclusions. This study establishes qPCR as a robust tool for large-scale microbiome profiling, offering enhanced accuracy, sensitivity, and quantitative capabilities compared to standard NGS methods. Our findings advocate for the integration of qPCR in standardizing microbiome detection, providing a pathway towards developing human microbiome profiling platforms capable of accurate species quantification. The adoption of qPCR assays could lead to more consistent, reliable, and cost-effective microbiome research and diagnostics.

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NOVEL METHOD FOR PROBING HOST-PATHOGEN INTERACTIONS OF *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile (*C. difficile*) is an anaerobic, spore-forming intestinal pathogen resistant to antibiotic treatment. In the US alone, severe recurrent form of the infection caused by this bacterium (CDI) takes ~29,000 lives annually. Development of the novel antibiotic agents is hindered due to a lack of accessible anaerobic *in vitro* platforms, allowing for the modeling of the host-pathogen dynamics between *C. difficile* and mammalian host cells. To address this problem, our team has designed an accessible, high-resolution live-cell imaging method for spatiotemporal characterization of stringent response by *C. difficile* in real time. We have successfully developed a novel R20291 *C. difficile* line capable of expressing a red fluorescent reporter IFP2.0. When used in combination with our previously reported Rose Chamber imaging method, IFP2.0-tagged R20291 cells can be used to detect and quantify fluorescence signal resultant from the IFP2.0 expression under the pTet promoter. IFP2.0-tagged *C. difficile* line was used to characterize biofilm penetration by antibiotic agent in real time. The outcomes of the study provide a previously unreported baseline parameters for quantitative fluorescent analysis of IFP2.0 expression in *C. difficile*, as well as unique spatiotemporal information about the effectiveness of *C. difficile* biofilm against antibiotic treatment.

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Thursday, July 11 — Gut Microbiome

1530-1730 Poster Session II: Gut Microbiome

Posters will be presented in Poster Session II
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DELETION OF THE NOVEL LIPOPROTEIN N-ACYLTRANSFERASE IN *BACTEROIDES* ALTERS BACTERIAL PHYSIOLOGY AND INDUCES IL-10 PRODUCTION IN MACROPHAGES

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Bacteroidota account for nearly 50% of the human gut microbiota and dramatically influence host health. These bacteria degrade a variety of structurally diverse glycans provided from diet or derived from the host, in turn providing nutrients for gut inhabitants and releasing short-chain fatty acids (SCFAs) that benefit the host. Glycan degradation is facilitated by lipoproteins, globular proteins anchored to the cell surface and outer membrane vesicles (OMVs) by a lipidated N-terminal cysteine. Lipoproteins are involved in numerous other processes, including cell envelope integrity, nutrient uptake, and immunomodulation via Toll-like receptors (TLRs) 2, 1, and 6. Despite their importance, how lipoproteins are synthesized in *Bacteroides* is understudied. Herein, a library of *B. fragilis* genomic DNA was screened for the ability to rescue a conditional-lethal *E. coli* strain deficient in lipoprotein N-acylation. Results identified a single protein, Lnb, responsible for this same process in *Bacteroides* that is distinct from all currently known *N*-acyltransferases. When Lnb is deleted, cells are less viable, filamentous, and show altered growth on polysaccharides. Proteomics studies on membranes and OMVs produced by Lnb- cells revealed significant changes in protein composition and localization. Initial studies in bone-marrow derived macrophages demonstrate increased production of the anti-inflammatory cytokine IL-10, but not IL-6, when exposed to live Lnb- cells. Taken together, these results highlight the importance of lipoprotein acylation, mediated by the novel Lnb acyltransferase, in *Bacteroides* physiology and its potential impacts on the host immune response.

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BIFIDOBACTERIA DEMONSTRATE MULTIPLE STRAIN-SPECIFIC MECHANISMS TO PROTECT AGAINST INFLAMMATION-INDUCED BARRIER DISRUPTION IN A PRIMARY HUMAN COLONOID MODEL

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Understanding the mechanisms underlying host-microbiome relationships has both potential to advance microbiome-targeted therapeutic strategies, as well as the design of novel live biotherapeutic products. Bifidobacteria are consistently health-associated commensal microbes utilized in commercially available probiotic products; however, the mechanisms by which Bifidobacteria promote homeostasis in the gut are not well understood. To this end, we have developed a primary human 2D colonoid system using asymmetric oxygen conditions well suited for co-culture of oxygen sensitive microbes with intestinal epithelial monolayers. These polarized monolayers have accessible apical and basolateral surfaces that can be differentially exposed to stimuli including microbes, microbial metabolites, and cytokines. Using a model of inflammatory cytokine-induced barrier disruption, we have begun to evaluate the ability of Bifidobacteria strains to protect the intestinal epithelium using metrics, such as transepithelial electrical resistance (TEER) and permeability of small molecules (FITC dextran translocation). We have shown that the metabolite indole lactic acid (ILA), produced by Bifidobacteria in a strain-specific manner, is able to delay inflammatory cytokine-induced reduction in TEER values, as well as prevent increased barrier permeability. Furthermore, human-derived *Bifidobacterium adolescentis* strain 269-1 showed a similar protection against increased barrier permeability, using both live culture and bacteria-free conditioned media. Further experiments will characterize more bacteria-host interactions from our human-derived strain collection, as well as pursue untargeted metabolomics analysis to generate candidate molecules that may be mediating this effect.

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HEMIN'S ROLE IN COMPETITION BETWEEN BACTEROIDES AND PREVOTELLA IN THE GUT MICROBIOME

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Diet plays a pivotal role in shaping the composition and activity of the gut microbiome, thereby having the potential to impact human health. Prevotella and Bacteroides are two major bacterial genera in the human gut microbiome that have been linked to certain dietary habits. Populations of Prevotella typically thrive in plant-rich diets, while Bacteroides populations are typically more abundant with higher consumption of meat. Understanding the Prevotella/Bacteroides ratio in the gut microbiome is crucial due to its implications for human health and disease. For instance, a lower Prevotella/Bacteroides ratio has been observed in individuals with obesity and inflammatory bowel disease. Here, we demonstrate different responses of *Prevotella copri* DSM 18205 and *Bacteroides uniformis* ATCC 8492 to varying hemin concentrations, a factor that is required for the growth of many anaerobic bacteria and that is abundant in red meat. Our findings show that *P. copri* DSM 18205 exhibits enhanced growth rate at a high hemin concentration (35µM) compared to *B. uniformis* ATCC 8492. Conversely, at low hemin concentrations (below 7µM), *B. uniformis* ATCC 8492 outcompetes *P. copri* DSM 18205 in growth rate. This result is inconsistent with the observation that a low Prevotella/Bacteroides ratio is linked to high meat consumption. We are currently investigating other dietary factors that might modulate the Prevotella/Bacteroides ratio in the gut microbiome. Advancing our understanding of the interplay between diet and the Prevotella/Bacteroides ratio will provide valuable insights for informing strategies to manipulate the gut microbiome and improve human health.

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CUTIBACTERIUM ACNES DIFFUSIBLE MOLECULES AFFECT *STAPHYLOCOCCUS LUGDUNENSIS* BIOFILM FORMATION AND ADHESION TO HOST EPITHELIAL CELLS

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Cutibacterium acnes, a major commensal of the human skin microbiome, has been shown to protect the skin against certain pathogens. In previous work, we investigated whether molecules produced by *C. acnes* could affect the biofilm formation of *Staphylococcus* species. We have seen that these molecules have antibiofilm activity against *Staphylococcus lugdunensis*, a common human skin commensal, without affecting their planktonic growth. In the present study, we show that different *C. acnes* strains produce molecules with antibiofilm activity against *S. lugdunensi*s in a dose-dependent manner. We also showed that these molecules affect the initial steps of *S. lugdunensis* biofilm formation, while significantly affecting *S. lugdunensis* colony morphology. The impact of these molecules was evaluated in human epithelial cells, and we observed that they did not impact the metabolism of these cells, indicating that they have no negative impact in their viability. We also assayed for the ability of these molecules to inhibit adhesion of *S. lugdunensis* in A549 epithelial cells, and we observed that they inhibited *S. lugdunensis* adhesion to these cells. *C. acnes* genome was sequenced, and three genetic clusters associated with secondary metabolism were found. We compare these regions to other publicly available genomes for three *C. acnes* strains that also showed antibiofilm activity, and these strains possessed at least two of the three biosynthetic gene clusters found in our original strain. Therefore, these genetic clusters present themselves as candidates involved in antibiofilm activity. Transcriptomics of *S. lugdunensis* was performed by RNASeq and revealed genes that were differentially expressed in the presence of *C. acnes* diffusible molecules. Finally, *C. acnes* diffusible bioactive molecules were characterized as resistant to boil, smaller than 3KDa and soluble in ethyl acetate and butanol. We fractionated the supernatant by High Performance Liquid Chromatography (HPLC) and obtained a fraction with significant antibiofilm activity. Understanding the interactions between different microorganisms could shed light on new compounds with potential applications to help treat and prevent bacterial infections.

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ANAEROBE-DERIVED SHORT-CHAIN FATTY ACIDS DISRUPT LIPID MEMBRANE HOMEOSTASIS IN *STAPHYLOCOCCUS AUREUS*

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The accumulation of mucus in the airways of people with cystic fibrosis (CF) or chronic rhinosinusitis (CRS) leads to hypoxic airway environments that are often colonized by pathogenic bacteria and aspirated oral anaerobes like *Streptococcus*, *Fusobacterium*, and *Prevotella*. Many of these anaerobes can degrade mucins and metabolize their constituent saccharides and amino acids, releasing fermentation byproducts like short chain fatty acids (SCFAs). Despite their prevalence and abundance, how these anaerobes and their metabolites contribute to disease or influence the behavior of canonical CF/CRS pathogens like *Staphylococcus aureus* remains poorly understood. To address this, we characterized the response of *S. aureus* grown in media with the SCFAs propionate and butyrate, both of which impaired growth and lead to a significant reduction in membrane potential. Transcriptomic and proteomic analyses identified branched chain amino acid (BCAA) and branched chain fatty acid (BCFA) metabolism as being induced by SCFAs. BCAAs are important substrates for BCFAs, the major lipid species in the *S. aureus* membrane. Supplementation of growth medium with the BCAA isoleucine partially restored *S. aureus* growth in the presence of SCFAs, and a targeted genetic screen of mutants in BCAA and BCFA metabolism identified several mutants that fail to display isoleucine-enhanced growth when SCFAs were present. Targeted lipidomics confirmed that the relative abundance of BCFAs was significantly reduced by propionate or butyrate in the medium. We show that altered BCFA metabolism disrupts the AgrBDCA quorum sensing system that regulates several important virulence factors. Finally, SCFAs sensitize *S. aureus* to the membrane-targeting antibiotics colistin and daptomycin, and propionate modestly reduces its fitness in co-culture with the major CF pathogen *Pseudomonas aeruginosa*. Together, these data show that *S. aureus* physiology in the CF/CRS airways may be significantly impacted by the composition of the mucosal microbiota and their metabolic milieu. Future work further dissecting the specific *S. aureus* targets of SCFAs are needed, as well as how the nutritional environment of the CF/CRS airways selects for specific community states that influence *S. aureus* colonization and growth.

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EXAMINATION OF THE B-LACTAM/B-LACTAMASE COMBINATION RESISTANCE MECHANISMS OF *BACTEROIDES* SPECIES

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Statement of purpose: β-lactam/β-lactamase combination resistance is increasing among anaerobic bacteria, mainly in *Bacteroides*. In aerobic counterparts β-lactam/β-lactamase combination resistance usually arises by amino acid changes for Class A β-lactamases. In *Bacteroides,* there are some β-lactamases (CfiA, CfxA and PbbA) that can mediate β-lactam/β-lactamase combination resistance. In this study, we aimed to investigate in more detail what factor (which genes with what amino acid substitutions and gene activations) codes for β-lactamase combination resistance among *Bacteroides* strains.

Materials/methods: 31 *B. fragilis* group isolates were selected whose amoxicillin/clavulanate (fixed ratio) MICs has been already recorded by agar dilution in an earlier antibiotic susceptibility study. These were enriched by obtaining MICs by a fixed inhibitor concentration gradient method. We detected the *cepA*, *cfxA*, *cfiA* and *pbbA* genes by RT-PCR and sequenced and detected the 5' regions of the *cfxA* genes. We did conjugation experiments to transfer the resistance phenotype of some selected strains to a susceptible host.

Results: None of the strains carried the *cfiA* or the *pbbA* gene and all the *B. fragilis* strains carried the *cepA* gene. Of the 11 amoxicillin/clavulanate-resistant (fixed ratio) *B. fragilis* strains, 9 carried the *cfxA* gene, while from the 10 amoxicillin/clavulanate-susceptible (fixed ratio) *B. fragilis* strains none (p=0.0002). For the non-fragilis *Bacteroides* strains included in the study (4 resistant and 6 susceptible to amoxicillin/clavulanate-resistant at a fixed ratio) all but one were *cfxA* gene positive. In the *cfxA*-positive *B. fragilis* strains, the Tyr227 was usually variant, however, in the non-fragilis *Bacteroides* strains, the Tyr227 dominated. In the cases of the upstream regions of the *cfxA* genes, we found an opposite relation – for *B. fragilis,* it was mostly uniform (1.2 kb), but for the non-fragilis *Bacteroides* for the strains susceptible to amoxicillin/clavulanate and piperacillin/tazobactam, it was 1.2 kb contrary to the resistant ones where it was mutated (3 kb, p=0.047 for non-fragilis *Bacteroides*). Interestingly, the β-lactam/β-lactamase combination resistance was not transferable in conjugation experiments for at least 3 studied strain.

Conclusions: As summary, we can say that mainly the *cfxA* genes are responsible for β-lactam/β-lactamase inhibitor combination resistance in *Bacteroides* and *B. fragilis* and non-fragilis *Bacteroides* strains differ in the possible manifestation - amino acid change or activation mechanism.

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BIFIDOBACTERIA LEVERAGE TYPE IVA PILI TO CAPTURE RESISTANT STARCH AND ENHANCE THEIR ABILITY TO METABOLIZE AN INSOLUBLE SUBSTRATE

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The composition and activity of the gut microbiome are largely influenced by dietary fibers, including Resistant Starch (RS) – a plant polysaccharide that evades degradation in the small intestine but can be metabolized by specialized bacteria in the colon. However, our understanding of how bacteria in the turbulent gut environment capture insoluble substrates like RS remains limited. In our study of 140 healthy participants who supplemented their diets with RS, Bifidobacteria were found to be the most responsive bacteria, constituting up to 24% of the relative abundance. To further investigate these bacteria, we isolated Bifidobacteria from fecal samples to assess their degradation of RS. We observed that cultivars of Bifidobacteria flocculate starch granules due to their attachment to RS granules via a pilus filament. When grown on RS or maltodextrin – a subunit of RS – *B. pseudocatenulatum* 851 induced the expression of a cluster of genes corresponding to a Type IVa sortase-dependent pilus. Upon shearing the pili from B. *pseudocatenulatum* 851 via blending, the cells lost the ability to adhere to RS granules, prompting us to investigate whether these pili enhance their ability to metabolize RS. Similar pilus genes were found in metagenomes from our interventional dietary study associated with multiple *Bifidobacterium* species that responded to RS. Additionally, related pilus gene clusters were identified in published metagenomes from people worldwide, suggesting that these pili are often involved in interactions between diet and the human gut microbiome.

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SNEATHIA VAGINALIS INVADES AMNIOTIC EPITHELIAL CELLS

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Sneathia vaginalis is a highly fastidious Gram-negative bacterial anaerobe and a component of the vaginal microbiome that was grossly under-recognized prior to the widespread use of culture-independent, DNA-based methods of detection. Within the past decade, DNA sequencing has revealed that it is a leading species detected in amniotic fluid and is consistently associated with preterm birth and preterm premature rupture of membranes (PPROM). We have reported on the cytopathogenic toxin A (CptA), a pore-forming toxin produced by *S. vaginalis*, but its biology and virulence determinants currently remain very poorly understood. PPROM, or membrane rupture in the absence of contractions prior to 37 completed weeks of gestation, is caused by preterm weakening of the membranes, particularly the amnion, which is primarily responsible for the tensile strength of the amniotic sac. However, the causes of pathologic deterioration leading to PPROM are not completely understood. We reported previously that *S. vaginalis* is able to traverse fetal membranes, and the **Purpose** of this study was to begin to characterize the effects of *S. vaginalis* on amnion through an investigation of its ability to invade amniotic epithelial cells. **Methods:** amniotic epithelial cells were isolated from term placentas from healthy pregnancies and cultured *in vitro*. Monolayers were challenged with *S. vaginalis* strain SN35 in mid-exponential log phase and detection of invasion was accomplished by both confocal microscopy and gentamicin protection assay. Actin microfilaments and microtubules were observed by confocal microscopy as well. Results: SN35 efficiently invaded the amniotic epithelial cells. Invasion did not affect the actin cytoskeletal structure but caused rapid disassembly of microtubules. **Conclusions:** *S. vaginalis* actively invades amniotic epithelial cells and disrupts microtubules. Intracellular invasion could play an important role in the pathogenesis of this species.

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METABOLOMICS OUTCOMES AS A FUNCTION OF SAMPLE COMPLEXITY FROM COLORECTAL CANCER-RELATED BACTERIAL STRAINS

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With colorectal cancer (CRC) cases rising worldwide, there is an increasing need for non-invasive CRC screening and detection of alternative biomarkers from complex biological samples. Current methods fail to provide accurate identification and abundances of compounds from complex clinical samples including fecal samples analyzed by mass spectrometry (MS). This work aims to investigate how the changing complexity of MS samples impacts the characterization of metabolites and the results of untargeted metabolomics. We performed untargeted MS on CRC-relevant and non-CRC-relevant bacteria of the human gut microbiome each cultured individually. To generate additional complexity, we created an MS sample of a mixture of all monoculture extracts combined. Finally, we co-cultured each of the CRC-related bacterial strains to determine if bacterial interactions generated additional complexity. Preliminary results indicated a higher number of metabolites in the complex samples as compared to the monoculture samples. Our analysis methods detected shared metabolites between the monoculture extracts and the complex mixture of all monoculture extracts, yet metabolite abundances were more variable in this complex sample. In addition, co-culture of bacterial strains did increase MS complexity, generating unique features that were not found in monoculture. Overall, we show that sample complexity can obscure ion detection and accurate abundance measurement of metabolites, although our untargeted MS analyses of these complex anaerobic bacterial extracts did reveal many unknown molecules poised for further identification. Accurate analysis of complex biological samples can identify novel biomarkers for diseases like CRC and increase the metabolic information gathered from approaches such as untargeted MS.

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INVESTIGATING THE CARBAPENEM RESISTANCE MECHANISM OF A *PHOCAEICOLA DOREI* STRAIN

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Statement of purpose: Earlier, we isolated an imipenem resistant *P. dorei* strain which did not carry the usual *cfiA* or *crxA* genes described for carbapenem resistance of *Bacteroides* spp. We aimed to determine its resistance mechanism by molecular methods.

Methods: We applied next generation sequencing, susceptibility measurements using gradient methods, β-lactamase activity test, molecular cloning, plasmid isolation, RT-PCR for gene detection and copy number and expression determination, as well as conjugal transfer.

Results: The genome of the *P. dorei* 2070 strain was around 5.2 Mb (in 201 contigs) by Illumina sequencing, and it contained two β-lactamase genes, *cfxA*2070 and *bla*HGD1. Its *cfxA* gene was a novel variant with three amino acid substitutions (L140F, T202I and H237R), but the *bla*HGD1 gene has been turned out to be harbored by all tested *P. vulgatus/dorei* strains. The cellular lysate of the strain yielded a > 1000 u/mg β-lactamase (nitrocefin) and a ca. 3.2 u/mg carbapenemase activity. Cloning the *cfxA1*, *bla*HGD1 and *cfxA*2070 alleles into a *B. fragilis* susceptible host showed that expression of the *cfxA*2070 allele can cause an 0.125 µg/ml MIC value (4-fold increase compared to the vector, 0.032 µg/ml). RT-PCR, copy number (15-30-fold increase) and plasmid isolation experiments (2.7, 4.1, 10 and 12 kb) suggest that an excised and amplified copy of the parental MTn4555 transposon is responsible for the high β-lactamase activities.

Conclusions: The *cfxA*2070 allele with its amplification on the MTn4555 transposon can also cause carbapenem resistance in *P. dorei*. This also warns that the non-fragilis carbapenem resistance among *B. fragilis* group species may also be significant.

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COMPARATIVE UNTARGETED METABOLOMICS ANALYSIS OF *BACTEROIDES FRAGILIS* STRAINS: IMPLICATIONS FOR COLORECTAL CANCER

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Colorectal cancer (CRC) is the second highest cause of cancer-related deaths worldwide and a major global health concern. As understanding of CRC grows, it is becoming increasingly evident that the gut microbiota, harboring trillions of bacteria, plays a substantial role in CRC development and progression. *Bacteroides fragilis*, a prominent member of the gut microbiota, has been implicated in both health and disease. *B. fragilis* is usually considered as a commensal organism that is part of the normal microbiota of the human colon. However, several *B. fragilis* strains secrete an enterotoxin known as the *B. fragilis* toxin (BFT) that may have a role in CRC development and progression. This study aims to elucidate the *in vitro* metabolomic differences between two *B. fragilis* strains – enterotoxigenic *B. fragilis* (ETBF) associated with CRC and nontoxigenic *B. fragilis* (NTBF) unrelated to CRC – to gain insights into their potential roles in colorectal carcinogenesis. Untargeted metabolomics was employed to comprehensively analyze the small molecule metabolomic profiles of these two strains. Preliminary results revealed distinct metabolic signatures between NTBF and ETBF, suggesting potential contributions to the CRC-associated phenotype of ETBF. The observed differences in metabolite profiles between these two strains underscore the potential role of some *B. fragilis* strains in CRC pathogenesis. These findings provide valuable insights into the metabolic adaptations of *B. fragilis* strains in the context of CRC, highlighting potential targets for further investigation and therapeutic interventions.

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AN IMPROVED METHOD OF MOUSE FECAL PREPARATION FOR GAVAGE IN GERM-FREE MICE

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Commensal gut microbiota plays a pivotal role in the host protection against many enteric pathogens. Conventional and germ-free mice are used extensively as a model to study the gut microbiota. However, the most effective method of microbiota preparation to capture the entire fecal microbiome consortium for colonization in germ-free mice are not known. Mice produce a limited amount of feces with a high amount of insoluble material, which interferes with subsequent procedures. Fecal suspensions are usually subjected to low-speed centrifugation to sediment insoluble particles. We showed that low speed centrifugation also resulted in pelleting of some gut bacteria, which clumped in the fecal suspension. Using microbiology, next-generation sequencing, and bioinformatics analysis, we optimized the parameters for fecal preparation, and generated a sufficient amount of suspension for gavage from a small amount of fecal material. This approach yielded gavage samples that were relatively free from insoluble suspended particles. Moreover, 16S rRNA sequencing analysis demonstrated that more species were recovered from samples prepared using the improved method, which should increase the efficiency of microbiome transfer to germ-free mice.

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NOVEL NON-ATHEROGENIC BREAKDOWN OF CHOLINE AND CARNITINE BY A GUT BACTERIUM: *CITROBACTER AMALONATICUS* CJ25

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Gut microbiota and human physiology are closely associated with each other and gut microbiota can influence disease through immunological and metabolic activity. Gut metabolism of quaternary amines (QAs), such as choline and carnitine has been shown to influence cardiovascular as well as renal health in an individual. Choline is cleaved by a glycyl radical enzyme choline TMA Lyase (*CutCD*) (2) and carnitine is cleaved by enzymes of the *caiABCD* operon followed by enzymes of the *bbu* operon in the human gut (3). The breakdown of QAs yields trimethylamine (TMA), a metabolite known to influence cardiovascular and renal health in individuals. TMA in the gut travels to the liver and is further oxidized to TMAO by the FMO3 enzyme, this accumulation of TMAO plays a critical role in causing atherosclerosis in humans. Interestingly, CJ25, the strain isolated and characterized by our lab has been shown to grow on choline, but does not encode canonical *CutCD* enzymes or any known methyltransferases from COG5598 superfamily that are known to demethylate QAs. Since the genome does not have cutCD or known methyltransferases, we tried to decipher the choline and carnitine metabolism using a combined metabolomics and proteomics approach. The metabolomics showed us significant accumulation of glycine betaine (GB) and the proteomics showed putative dehydrogenases that could be performing sequential oxidation of choline or carnitine to GB. We *hypothesize* that CJ25 is using dehydrogenase homologs to break down choline and carnitine into the non-atherogenic product, GB. The discovery of this putative non-atherogenic pathway will establish CJ25 as a potential probiotic agent in the human gut. Furthermore, these putative initial pathways for choline and carnitine breakdown may also exist in other gut microbiota, which could amplify the effects of these pathways significantly and possibly reduce the risk of atherosclerotic cardiovascular disease.

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AKKERMANSIA MUCINIPHILA, METHANOBREVIBACTER SMITHII, AND *PREVOTELLA* SPP. IN INTESTINAL MICROBIOTA OF PATIENTS WITH MULTIPLE SCLEROSIS IN TURKEY: A QUANTITATIVE COMPARISON WITH HEALTHY INDIVIDUALS

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Aim: In the sources *A.muciniphila*, *M.smithii*, and *Prevotella* spp. of intestinal microbiota are suggested to be associated with MS. We aimed to investigate their amount in the gut microbiota of the Turkish population with MS.

Materials and Methods: The study was carried out between the years 2022-2023 with stool samples of 40 treated, 40 untreated MS patients, and 40 healthy individuals. The relative abundances of *A.muciniphila*, *M.smithii*, and *Prevotella* spp. to the total amount of bacteria in the intestinal microbiota were investigated by real-time PCR and data were compared statistically.

Results: The levels of *A.muciniphila* in both treated and untreated MS patients was lower compared to healthy individuals ($p<0.001$, $p<0.001$). Comparing with healthy women and men these lower levels were observed in treated and untreated MS women and in treated MS men ($p=0.002$, $p<0.001$, $p=0.023$). *M.smithii* levels were significantly reduced in untreated MS patients, especially in untreated women versus healthy controls ($p=0.004$, $p=0.010$).However, a significant *M.smithii* increase was observed in treated women compared to treated men and untreated women ($p=0.011$, $p=0.028$). Furthermore, *Prevotella* spp. levels were decreased in treated and untreated patients compared to controls ($p=0.002$, $p=0.001$).

Conclusion: *A.muciniphila*, *Prevotella* spp., *M.smithii* in the intestinal microbiota of MS patients in our country were found lower than those of healthy individuals. Treatment did not significantly alter these bacterial levels, except for the archaeon *M. smithi*i which was increased in MS women. We believe that new perspectives on the course and treatment of MS may be developed explaining the decrease in the amount of these bacteria through microbiome analysis.

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UNDERSTANDING CARBON AND NITROGEN METABOLISM BY HUMAN GUT BACTEROIDOTA AND DEVELOPING GENETIC TOOLS FOR UNDERSTUDIED MICROBES

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Human gut Bacteroidota are known to metabolize a diverse array of carbon substrates in their native environment, but due to a lack of tools, these capabilities have not been studied before with high throughput screens on a diverse group of microbes. Here, we developed multiple barcoded transposon libraries in diverse species and carried out large scale functional genetics screens to understand their carbon and nitrogen metabolisms.

First, we developed new tools to be able to generate large scale transposon mutant libraries in various genera in this phylum using a magic pools approach, which involves developing a library of transposon delivery vectors to identify one that works for specific strain. We used a RnaSeq guided approach to identify strong promoters from native strains and codon usage data to codon optimize both the drug selection markers and the transposase protein.

Second, we developed barcoded transposon mutant libraries for *Bacteroides ovatus, Bacteroides finegoldii, Bacteroides stecoris, Phocaeicola vulgatus* and *Phocaeicola dorei* and carried out functional genetics screens in hundreds of carbon and nitrogen sources. We found novel genes and gene clusters involved in phosphosugar metabolism, sialic acid, and serine metabolisms. Using techniques such as genome integration and CRISPR, we were able to validate our findings and show both necessity and sufficiency for metabolisms of diverse phosphosugars. We also found phosphotases that had specificity based on the location of the phosphate group in a hexose sugar. At the same time, we were also able to map various PULs (polysaccharide utilization loci) to substrates.

Overall, this work has been super beneficial for understanding functions of genes in relatively understudied but prevalent and abundant human gut bacteria. This large-scale dataset can be used to develop better metabolic models for these organisms. We are very excited to test our tools on more gut Bacteroidota and in the future be able to do genetics screens on important microbes such as Prevotella which have been known to be more prevalent in traditional populations of humans.

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LARGE-SCALE GENETICS IN ANAEROBES LEADS TO ENZYMES DISCOVERY

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Chemogenomic fitness profiling of mutant bacterial strains are extremely powerful high throughput approaches which can enable evidence-based annotation of gene function. Using a randomly barcoded transposon-based approach (RB-TnSeq), we were able to gain knowledge in various aspects of the physiology of two anaerobic bacteria which are considered model organisms among soil microbes and gut microbes respectively. Using a wide range of growth conditions including respiration, fermentation, variation of nitrogen sources, nutrient input and exposure to stressors, we were able to generate large gene-phenotype maps that provide new insights into metabolism, regulation and stress response in these organisms. For instance, in the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough (DvH), we identified new enzymes involved in vitamin synthesis. The gene DVU0867 was shown to encode for an atypical L-aspartate decarboxylase which was missing from the annotated pantothenic acid synthesis pathway. We also discovered that the uncharacterized dehydrogenase DVU0826:DVU0827 is necessary for the synthesis of pyridoxal phosphate. Additionally, we provided the first experimental evidence that biotin synthesis in DvH occurs via a specialized acyl carrier protein, a dedicated set of fatty acids biosynthesis (FAB)-like enzymes, and without the formation and cleavage of methyl esters. In the gut commensal *Bacteroides thetaiotaomicron*, we explored the utilization of disaccharides, and we showed that the primary route of trehalose catabolism uses a 3-keto intermediate and identified the gene responsible for the hydrolysis of 3-keto-trehalose.

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SYNERGISTIC INTERACTION OF *AKKERMANSIA MUCINIPHILA* AND MUCIN-DEGRADING *BACTEROIDES* IN INFLAMMATORY BOWEL DISEASES

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Inflammatory bowel diseases (IBDs) are postulated to arise from a combination of host genetic susceptibility and environmental factors including diet and intestinal microbiota. We previously reported that feeding a fiber free diet to ex-germfree mice colonized with a synthetic microbiota containing 14 species (SM14) triggers lethal colitis when mice lack the IBD-associated cytokine, Interleukin-10 (IL-10). Il-10-/- mice colonized with a version of the synthetic microbiota lacking 4 species known to possess mucin-degrading abilities exhibited lower inflammation and significantly better survival, demonstrating a disease-promoting role of mucin-degrading bacteria. To test the roles of individual mucin degraders, we added them back to the synthetic microbiota either individually or in pairwise combinations. The presence of two mucin-degrading species (*Akkermansia muciniphila* and *Bacteroides thetaiotaomicron*) accelerated disease development with similar timing as the full SM14. Colonizing germfree IL-10-/- mice with just these two species also resulted in rapid disease development, suggesting that a combination of two species is both necessary and sufficient for rapid inflammation in this model. Another mucin-degrading *Bacteroides* (*B. caccae*) did not accelerate disease development as measured by survival when it was present within the community of non-mucin-degraders. However, when this species was present in the same community with *A. muciniphila,* it caused significantly decreased survival, albeit slightly longer time-to-death than observed with the full SM14 or the community harboring *Akkermansia muciniphila,* and *Bacteroides thetaiotaomicron* together. This work demonstrates that multiple combinations of mucin degrading bacteria are capable of acting synergistically during IBD pathogenesis and that diet and host genetic factors can unmask conditional pathogenic qualities of commensal gut anaerobes. Future work will focus on genetic analysis of the bacterial functions involved (e.g., mucin-degrading enzymes), mechanisms of synergy between mucin-degrading bacteria and determining if similar synergistic combinations occur in IBD patients that might contribute to disease occurrence.

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1530-1730 Poster Session II: Pathogenesis

Posters will be presented in Poster Session II
Thursday, July 11 1530-1730.

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CENTRAL *IN VIVO* MECHANISMS BY WHICH PROLINE REDUCTASE OF *CLOSTRIDIOIDES DIFFICILE* DRIVES EFFICIENT METABOLISM AND DISEASE PROGRESSION

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Clostridioides difficile (CD) is a toxin-producing nosocomial pathogen that opportunistically infects patients with a depleted gut microbiome that commonly occurs after antibiotic exposures. In colonizing the gut, CD preferentially metabolizes amino acids, with proline being a preferred reductive substrate that supports rapid energy generation and growth. We evaluated host outcomes in highly susceptible gnotobiotic mice infected with wild-type and Δ*prdB* mutants of the mouse-infective ATCC43255 strain to investigate how proline metabolism modulates CD's virulence, interactions with commensals, and host disease. While gnotobiotic mice infected with the Δ*prdB* mutant succumbed to infection, they demonstrated delayed colonization and toxin production, which extended their survival. *In vivo* metatranscriptomic analyses of monoassociated mice demonstrated broad shifts from Stickland amino acid fermentations to carbohydrate metabolism in the Δ*prdB* mutant. To investigate functions of CD's proline reductase in interactions with the commensal microbiota, we evaluated infection dynamics in mice co-colonized with the disease-promoting commensal *Clostridium sardiniense* (CSAR). Delayed CD germination, colonization, and toxin B production were observed in the CSAR and ΔprdB-infected mice. Co-colonized mice with the wild-type strain rapidly succumbed within 48h, while 31% of CSAR and Δ*prdB*-infected mice survived. *In vivo* metatranscriptomic analyses from co-colonized mice identified additional metabolic defects in the Δ*prdB* mutant including failure to recruit oxidative Stickland pathways, and ornithine fermentations to harness CSAR-produced ornithine, effectively converting the mutant into a glycolytic strain that was now in direct competition with CSAR for carbohydrate substrates. CSAR remained metabolically active during the Δ*prdB* infection through mucin degradation and disaccharide and polysaccharide metabolism. Our findings illustrate central functions of CD's proline reductase in coordinating early metabolism to facilitate gut colonization, including interactions with cross-feeding commensal species.

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BIOINFORMATIC ANALYSIS OF *CLOSTRIDIOIDES DIFFICILE* COLLAGEN-LIKE BclA SPORE PROTEINS

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Clostridioides difficile is a Gram-positive, anaerobic spore-producing bacteria frequently implicated in antimicrobial-associated infections (*C. difficile* infections, CDI). *C. difficile* spores are essential for the transmission of the disease, which clinical symptoms range from mild diarrhea to severe pseudomembranous colitis, having a mortality rate of 9.3% and an alarming recurrence incidence of 20%. The outermost layer of the spore possesses collagen-like proteins (BclA1, BclA2 and BclA3) resembling hair-like projections. Previous work has demonstrated that the absence of BclA1 is associated with increased spore germination and impaired *in vivo* colonization efficiency. BclA3 protein has proven to be essential for hair-like projection formation and to contribute to pathogenesis, persistence, and recurrence of CDI. Additionally, *C. difficile* spores that lack any BclA protein show compromised exosporium assembly and decreased spore hydrophobicity rates. Despite the potential contribution to host-pathogen interactions and virulence that BclAs might confer to *C. difficile* spores; their prevalence and variability remains unclear. To investigate this matter, more than 20,000 *C. difficile* genomes were downloaded and used to generate a core-genome phylogenetic tree that displayed the complex organization of classical clades (C1 to C7) and cryptic clades (C-I to C-VII) that this pathogen is divided into. Subsequent *bclA* locus search and analysis revealed a high variability of the central collagen-like region (CLR) of the proteins, indistinctively of the clade the belonged to. Interestingly, one fourth of the genomes presented the exact same *bclA1* pseudogenization event all grouped on the same hypervirulent clade. A conserved pseudogenization was not observed for *bclA2* or *bclA3*. The extensive diversity of BclA alleles, mainly attributed to their CLR, and prevalence of BclA1 truncation may impact the pathogenesis of different *C. difficile* strains.

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VISUALIZING TOXIN GENE EXPRESSION DURING MURINE INFECTION IN *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile is an anaerobic, spore-forming, bacterial pathogen that is the most common cause of healthcare-associated infections in the United States. *C. difficile* is transmitted by spores through the fecal-oral route, which upon reaching the colon, germinate into metabolically active vegetative cells that produce toxins that damage the intestinal epithelium. Despite toxins being essential for causing damage to the host, toxin levels and *C. difficil*e biomass measured in feces during murine infection do not correlate with disease severity. These findings indicate our understanding of the factors determining disease severity during *C. difficile* infection (CDI) is limited. Previous work suggests that *C. difficile* exists in two subpopulations during murine infection, with most found in the gut lumen and a smaller subset located near the colonic epithelium. Recent analyses suggest that this epithelium-proximal sub-population may modulate disease severity and thus explain the lack of correlation between toxin levels and biomass in feces and clinical outcomes. With the role of epithelium-associated populations of *C. difficile* during CDI needing to be clarified, my work is focused on testing the hypothesis that the spatial distribution of *C. difficile* within the colon is a critical factor in determining disease severity. To address this hypothesis, I have constructed and optimized various fluorescent reporter strains to visualize *C. difficile* and toxin-specific gene expression during murine infection. Additionally, I have created reporter strains in toxin-null (ΔtcdR) and toxin over-producing (ΔrstA) strains to assess the impact of toxin production on *C. difficile* localization during infection. My work provides previously unknown information about the fitness of ΔrstA strains during murine infection, the spatial distribution of *C. difficile* as a determinant of disease severity, and the impact of toxin production on *C. difficile* localization in the colon during murine infection. Additionally, it demonstrates the ability to visualize toxin gene expression at the single-cell level during infection.

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IMPACTS OF *CLOSTRIDIUM PERFRINGENS* TOXINOTYPES ON ADHERENCE TO INTESTINAL MUCINS AND EPITHELIAL CELLS

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Clostridium perfringens has been associated with various significant systemic and enteric diseases in both humans and livestock, including gas gangrene, food poisoning and nonfoodborne diarrhoea, enteritis/enterocolitis, and enterotoxemia. The significance of toxins for the pathogenicity of *C. perfringen*s is clear because specific toxinotypes are linked to different diseases with host specificity. We hypothesized that difference in host epithelium adherence between *C. perfringens* toxinotype would contribute to host tropism. Strains of toxinotypes A, C, E, and F were investigated for their ability to adhere to the human intestinal cell line, Caco-2. The average percentage of adherence of the toxinotypes C (22.48%) and F (17.29%) was higher than type A (10.14%) , although the efficiency of binding to Caco-2 cells within toxinotypes was different depending on the tested strains. The presence of porcine gastric mucin on surface of Caco-2 cell or adhered to glass coverslips did not enhance the binding of tested *C. perfringens* type A strains. Future work will investigate adherence of additional strains from different toxinotypes to different epithelial cell lineage, as well as investigating potential molecular mechanisms underlying difference of adherence within and among toxinotypes.

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FLAGELLAR SWITCH INVERTED REPEAT SEQUENCE VARIATION IMPACTS *CLOSTRIDIOIDES DIFFICILE* RT027 VIRULENCE

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Clostridioides difficile (*C. difficile*) BI/NAP1/027 strains have been associated with more severe disease and increased toxin production. However, the ability of these strains to cause severe diarrhea and colonic inflammation varies from asymptomatic to lethal. To gain insight into the mechanisms that underly this differential virulence, we conducted a genomic and phenotypic comparison of a panel of RT027 isolates. While we observed a weak correlation between increased flagellar expression and virulence, consistent with previous reports, we determined that the flexibility of the flagellar switch is a key contributor to *in vivo* virulence. *C. difficile* flagellar expression is regulated through phase variation via site-specific DNA recombination. The DNA recombinase RecV recognizes the upstream switch of the *flgB* operon and reversibly inverts the switch sequence, leading to ON or OFF orientations for flagellar gene expression. Analysis of flagellar switch sequences identified variant inverted repeat (IR) sequences, with 40% of isolates having lost one A or T in the IR compared to the genome of virulent *C. difficile* R20291. Isolates with the same R20291 IR sequence exhibited increased switching flexibility (with 50%-70% ON) and virulence compared to those with variant IRs (with > 99% either ON or OFF). Moving the RecV and flagellar IR into a heterologous host, *E. coli*, recapitulated the impact of IR variants on RecV-dependent recombining efficiency. Taken together, our results suggest that specific IR types restrict the capacity of *C. difficile* RT027 isolates to invert the flagellar switch and decrease phenotypic heterogeneity during infection, reducing *in vivo* virulence. Our findings may help explain the *variable* disease outcomes observed in patients.

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DIETARY SUCROSE INDIRECTLY ENHANCES *CLOSTRIDIOIDES DIFFICILE* PATHOGENESIS

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Added sugars constitute about 13% of daily caloric intake in an average American diet, with 30% of Americans consuming excessive amounts of sugar. The impact of these dietary sugars on the pathogenesis of *Closridioides difficile*, a common enteric pathogen, remains unclear. Our study used a diet high in sucrose, a sugar commonly consumed but not metabolized by *C. difficile*, to investigate its effect on *C. difficile* infection (CDI). We monitored mice fed this diet for factors such as colonization, bacterial and toxin burden, clinical signs of disease, and tissue damage, following infection with *C. difficile*, with or without antibiotic pre-treatment. Our observations revealed that the high-sucrose diet increased susceptibility to CDI, worsened disease symptoms, and elevated toxin levels. Mice on this diet also maintained significantly higher levels of *C. difficile*, and struggled to clear the infection, even months post-infection. This led to a higher susceptibility to symptomatic relapse. Interestingly, even without the main CDI susceptibility factor, antibiotic pre-treatment, the high-sucrose diet made mice more vulnerable to *C. difficile* colonization. Our findings suggest that dietary sugars can significantly enhance *C. difficile* pathogenesis, even if not directly metabolizable by the pathogen. We hypothesize that this could be due to a combination of microbial and host factors, including changes in microbiome structure, metabolome profile, intestinal inflammation, and the innate immune response.

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CONSTITUTIVE VANG EXPRESSION ASSOCIATED WITH REDUCED *CLOSTRIDIOIDES DIFFICILE* FITNESS

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Use of vancomycin to treat *Clostridioides difficile* infection (CDI) has increased causing a selection pressure for resistance development. Vancomycin reduced susceptibility is associated with constitutive vanG expression. We aim to explore *vanG* expression to *C. difficile* fitness cost and patient outcomes.

This study included adult patients hospitalized with CDI in two health systems (14 hospitals) in the Houston area. CDI patients with treatment failure (continued diarrhea at day 14 or disease recurrence by day 30) were matched 1:1 to patients without treatment failure by recurrent disease, age +/- 10 years, and ribotype. Stool samples were cultured for *C. difficile*, ribotyped, and tested for vancomycin susceptibility by agar dilution. Quantitative reverse transcription PCR analysis was performed at baseline and after 15 minutes with either control or vancomycin exposure of 0.5 mg/ml. Transcript level analysis was completed using the ΔΔCT method and laboratory strain R20291 was used as the control. Growth curves were performed in duplicate over 24h using Cytation Imaging Reading. Analysis was performed on isolates with and without constitutive *vanG* expression using R including the Growthcurver package.

Twenty-six clinical isolates (13-matched pairs) from ribotypes RT027 (n=8), RT106 (n=8), RT002 (n=4), RT014-020 (n=4), and RT255 (n=2) were included. Vancomycin MIC range was 1–8 mg/L. Six isolates with constitutive vanG expression (defined as expression >100 fold change) had significantly lower mean area under the growth curve (AUC) than isolates without elevated *vanG* expression (7.6±2.52 vs 8.9±0.65, p=0.04). All isolates with constitutive *vanG* expression exhibited MICs >2 mg/L. 3 of 6 (50%) patients with constitutive expression strains experienced poor outcomes compared to 8 of 20 (40%) patients without *vanG* expression.

Constitutive *vanG* expression was associated with reduced *C. difficile* fitness, perhaps explaining its low occurrence in the community. However, patients with constitutive *vanG* expression may experience poorer outcomes than those without constitutive *vanG* expression.

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CHARACTERIZATION OF GENETICALLY MODIFIED *CLOSTRIDIUM SPOROGENES* AND ITS IMPACT ON GUT BILE ACID BIOTRANSFORMATIONS

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Clostridioides difficile is a Gram-positive, spore forming, anaerobic bacterium that causes *C. difficile* infection (CDI), which affects the colon and causes a range of clinical disease from diarrhea to pseudomembranous colitis, and even death. The gut bacteria provide colonization resistance through nutrient competition and making inhibitory secondary bile acids. Few strains of commensal Clostridia encode a bile acid inducible (*bai*) operon, that converts primary bile acids like cholate (CA) to secondary bile acids like deoxycholate (DCA) through 7-α dehydroxylation, and many are genetically intractable. Recent work has cloned the bai operon into *C. sporogenes* (MF001), creating a strain that we can use to study the contribution of both nutrient competition and bile acid modifications with genetic control, however they are not well characterized. We sought to characterize WT and MF001 strains, validating the maintenance of the *bai* gene plasmids over 72 hr. We measured the growth kinetics of both strains with and without CA, DCA, and iso-DCA and determined the minimum inhibitory concentrations (MICs). Using *baiF*, *baiG*, and *baiH* specific primers to screen for each plasmid, PCR and gel electrophoresis showed that MF001 maintained *bai* plasmids for up to 72 hr. MF001 supplemented with CA had a rapid decline in growth in CFUs compared to WT after 48 hr. MICs of CA for WT and MF001 were 10 mM. The MIC of DCA differed between the WT and MF001, and was 1.25 mM and 0.625, respectively. The inhibition of growth with MF001 in CA suggests that the *bai* operon is active, and potentially creating an inhibitory amount of DCA and or isoDCA. Future directions include bile acid metabolomics to measure the conversion of CA to DCA and isoDCA over 72 hr. A gnotobiotic mouse model fed cholate rich diet with and without challenge with WT and MF001 will also be used to determine the CA to DCA and isoDCA conversion in the host. Fully characterizing this strain *in vitro* and *in vivo* will prove to be a valuable tool in mechanistically dissecting the interplay between *C. difficile*, nutrient competition, and bile acid biotransformation.

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OLFACTOMEDIN-4+ NEUTROPHILS EXACERBATE INTESTINAL EPITHELIAL DAMAGE AND WORSEN HOST SURVIVAL AFTER *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Neutrophils are key first responders to *Clostridioides difficile* infection (CDI). Excessive tissue and blood neutrophils are associated with worse histopathology and adverse outcomes, however their functional role during CDI remains poorly defined. Utilizing intestinal epithelial cell (IEC)-neutrophil co-cultures and a pre-clinical animal model of CDI, we show that neutrophils exacerbate *C. difficile*-induced IEC injury. We utilized single-cell transcriptomics to illuminate neutrophil subtypes and biological pathways that could exacerbate CDI-associated IEC damage. As such, we have established the first transcriptomics atlas of bone marrow (BM), blood, and colonic neutrophils after CDI. We found that CDI altered the developmental trajectory of BM and blood neutrophils towards populations that exhibit gene signatures associated with pro-inflammatory responses and neutrophil-mediated tissue damage. Similarly, the transcriptomic signature of colonic neutrophils was consistent with hyper-inflammatory and highly differentiated cells that had amplified expression of cytokine-mediated signaling and degranulation priming genes. One of the top 10 variable features in colonic neutrophils was the gene for neutrophil glycoprotein, Olfactomedin 4 (OLFM4). CDI elevated the number of OLFM4+ neutrophils in the colon, and OLFM4+ cells aggregated to areas of severe IEC damage. Compared to uninfected controls, both humans and mice with CDI had higher concentrations of circulating OLFM4; and in mice, OLFM4 deficiency resulted in faster recovery and better survival after infection. Collectively, these studies provide novel insights into neutrophil-mediated pathology after CDI and highlight the pathogenic role of OLFM4+ neutrophils in regulating CDI-induced IEC damage.

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UNVEILING NEUTROPHIL PARADOX: DISCREPANCY BETWEEN ANTICIPATED ROLE AND ACTUAL IMPACT ON *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Clostridioides difficile is the most common cause of nosocomial infections in the US. The bacterial toxins damage intestinal epithelial cells (IECs) and drive *C. difficile* infection (CDI) pathology. The release of inflammatory mediators from IECs leads to the recruitment of innate immune cells, including neutrophils, to the site of infection. Despite their anticipated role in combating the pathogen, neutrophils paradoxically exacerbate IEC injury, revealing gaps in our comprehension of how *C. difficile* withstands colonic neutrophilia to persist in the colon. This study delves into the impact of neutrophils on *C. difficil*e burden both *in vitro* and *in vivo*.

Analysis of historical samples from various mouse models of CDI revealed a lack of correlation between tissue neutrophil counts and luminal *C. difficile* burden. To corroborate this discordance, we used transgenic mice (PMNDTR) that allow for selective and inducible depletion of endogenous neutrophils. At 24hrs post *C. difficile* challenge, both iDTR+/+ Cre+ mice (neutrophil deficient) and iDTR+/+ Cre- mice (neutrophil sufficient) had similar pathogen burden. Additionally, under anaerobic *in vitro* conditions, mouse bone marrow-derived neutrophils did not impede the growth and viability of the *C. difficile* bacteria, despite evidence of phagocytosis. However, co-culture with differentiated human neutrophilic promyelocyte cell line HL-60 altered early *C. difficile* growth kinetics without affecting bacterial burden beyond 24 hours. These findings underscore a disparity between the perceived role of neutrophils and their actual impact on CDI. Future studies using human and murine neutrophils alongside genetically modified strains of *C. difficile* will contribute to the elucidation of mechanisms by which *C. difficile* evades infection-induced neutrophil onslaught.

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DEFINING MECHANISMS OF Q223R POLYMORPHISM-ASSOCIATED DIFFERENTIAL NEUTROPHILIA IN CDI

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Clostridioides difficile infection (CDI) is one of the leading nosocomial infections in the U.S., affecting about 500,000 individuals every year. Both the magnitude of CDI-induced leukocytosis and host genetic makeup can impact disease severity and outcomes. We have previously reported an interplay between a common single nucleotide polymorphism (SNP) in the gene for leptin receptor (LEPR) and peripheral blood white blood cell (WBC) count in CDI patients. We found that CDI patients homozygous for the mutant LEPR allele resulting from a change from Glutamine (Q) to Arginine (R) at the 223rd amino acid position (i.e., QQ/WT to mutant/RR) had roughly 5-fold increased risk of having peak WBC count of >20 x 109/L in the peri-diagnosis time frame. Other reports have shown that this degree of leukocytosis in the same time frame predicts worse CDI outcomes. To start defining the biological mechanisms underlying the differential leukocytosis associated with this LEPR SNP, we utilize mice that express the same SNP (i.e., QQ/WT and mutant/RR). Similar to patients, RR mice exhibited more WBCs in peripheral blood after infection with two different strains of *C. difficile* (VPI10643 and M7404). Since neutrophils play an important role in regulating CDI pathogenesis, we determined neutrophil numbers in different body compartments. Although prior to infection, both WT/QQ and RR mice had similar neutrophil numbers, after infection, RR mice had significantly more neutrophils in bone marrow, blood and colonic tissue. In particular, we found that a neutrophil population that expresses Olfactomedin-4 (OLFM4) was increased in RR mice. Other data from our lab has shown that activated OLFM4+ neutrophils aggravate *C. difficile* toxin-induced epithelial damage. As leptin-LEPR signaling plays a key role in the processes of granulopoiesis, apoptosis and cell trafficking, we now plan to determine if increased neutrophil numbers (both OLFM4+ and OLFM4-) observed after infection in RR mice can be due to enhanced production, reduced cell death and/or altered neutrophil distribution. Defining how a common SNP drives neutrophilia during CDI has the potential to identify novel host-directed CDI therapies.

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UNDERSTANDING THE ROLES OF TcdE AND TcdL IN TOXIN RELEASE IN *CLOSTRIDIODIES DIFFICILE*

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The nosocomial pathogen *Clostridioides difficile* produces two large toxins, TcdA (308 kDa) and TcdB (270 kDa). Although toxin effector functions in host cells have been extensively studied, little is known about how these toxins are released from the bacterium. We recently developed highly-sensitive reagents to detect TcdA and TcdB *in vitro* and *in vivo*. Using these reagents, we generated growth curves with a variety of different *C. difficile* strains and found toxin release varies greatly in log-phase, stationary phase, and death phase amongst strains. TcdA and TcdB are encoded on a pathogenicity locus 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 can promote lysis-independent toxin release. To further understand toxin secretion, we created deletions in tcdE and tcdL and found these strains released less toxin compared to the parent strain. We tested the relevance of these deletions *in vivo* and found both had significantly reduced disease severity when compared to infection with the wild-type strain. Next, we sought to investigate the mechanism of how TcdL contributes to toxin release. We found that strains lacking tcdL had thinner, more compact cell walls, and had less cell wall remodeling. In addition, these strains were more resistant to the beta-lactam, ampicillin. Our data indicate that both TcdE and TcdL are required for lysis-independent toxin release.

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IDENTIFYING SPORE-SURFACE LIGANDS INVOLVED IN E-CADHERIN-MEDIATED ADHERENCE OF *CLOSTRIDIOIDES DIFFICILE* TO INTESTINAL EPITHELIAL CELLS

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Attachment of *C. difficile* spores to intestinal epithelial cells has been shown to be facilitated by the novel spore adherence receptor, E-cadherin. Upon binding to E-cadherin, *C. difficile* spores are directed to adherens junctions, which is enhanced by TcdA/TcdB intoxication. Intriguingly, spores from *bclA* mutant strains, lacking a known binding protein, exhibited affinity for E-cadherin suggesting that unidentified spore surface ligands, distinct from BclA, are implicated in E-cadherin binding. Therefore, in this work we aim to identify and characterize novel spore surface ligands involved in E-cadherin-mediated adherence to IECs. For this, we employed 2-D Far-Western blotting of spore coat and exosporium extracts blotted against E-cadherin with which we identified several immunoreactive spots. Selected spots were trypsin-digested and subjected to mass spectrometry (MS/MS). Although numerous proteins were identified in each immunoreactive spot, we selected candidates whose expression is under the control of the sporulation-specific sigma factors, SigE or SigK. Currently, we are validating selected candidates by overexpressing in *E. coli* and performing Far-Western blotting. Subsequently, validated ligands will be studied for E-cadherin binding specificity using enzyme-linked immunosorbent assays (ELISA) and bio-layer interferometry (BLI). After identification and validation of spore surface ligand(s), the basis of the interaction will be explored by site directed mutagenesis, generating variants that fail to bind to E-cadherin while allowing fully assembly of the exosporium layer. Mutant variants will be further characterized for their impact in E-cadherin-mediated adherence to IECs. These experiments will be the first to implicate a spore surface ligand to E-cadherin binding and determine its role in spore adherence.

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MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) DYNAMICS IN *CLOSTRIDIOIDES DIFFICILE* INFECTION (CDI): KINETICS, SOURCES, AND DRIVERS

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Clostridioides difficile infection (CDI) is a nosocomial infection causing approximately 15,000 deaths annually in the U.S. Macrophage migration inhibitory factor (MIF) is a pleiotropic pro-inflammatory cytokine which is present in various cell types as pre-formed intracellular pools. Our lab has previously demonstrated that systemic MIF is elevated in CDI patients, and in mice with CDI, systemic and tissue MIF concentrations increase within 24 hours of infection. Further, blocking MIF before CDI reduced colonic damage and tissue neutrophils and improved survival, indicating its detrimental role in CDI pathogenesis. Together, our data suggest that MIF is an acute-phase cytokine with a deleterious role in CDI pathogenesis and raise the possibility that targeting this cytokine could have the potential as a host-targeted therapy to reduce CDI severity. However, the timing of peak MIF production in response to CDI, where anti-MIF intervention could be most impactful, and the critical cellular sources of CDI-induced MIF remain unknown. Using patient samples, we found the highest plasma MIF concentration on days -2 and -1 from diagnosis (i.e., the incubation period of CDI) with a subsequent decline in the days after diagnosis. In mice, we determined intracellular MIF levels in both immune and non-immune cells of colonic tissue in a similar time frame (i.e., on days 1, 2, and 3 after the *C. difficile* spore challenge) using high-dimensional flow cytometry. We found the highest percentage of MIF in cells of the innate immune system on day 2 after infection, whereas in intestinal epithelial cells (IECs), intracellular MIF peaked on day 3 after CDI. The mean fluorescence intensity of MIF expression was higher in eosinophils, neutrophils, and macrophages compared to IECs. In sum, our data provide novel insights into MIF kinetics in CDI and suggest that immune cells are a key producer of MIF during the early phase of the infection. Given that *C. difficile* toxins are the prominent virulence factors of CDI, we will next explore the effect of *C. difficile* toxins on MIF expression across various cell types *in vitro* and *in vivo* using different *C. difficile* strains (both toxin-deficient and sufficient).

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INHIBITION OF *CLOSTRIDIOIDES DIFFICILE* SPORULATION BY COMMENSAL AND PATHOGENIC ENTEROCOCCI

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Endospore formation by *C. difficile* is necessary for persistent and relapsing colonization of the gut. Gut clostridia, pathogenic and commensal, must integrate information from the environment when making the cell fate decision to form a dormant endospore. Thus, it is likely that metabolism by co-inhabitants of the gut can affect *C. difficile* sporulation. Here, we have determined that co-culture of *C. difficile* with most species of pathogenic vancomycin-resistant enterococci (VRE) and commensal enterococci (n = 10) lowers sporulation efficiency on solid and liquid growth medium, without significantly affecting vegetative growth of either organism. We have identified *Enterococcus saccharolyticus* as an exception that does not inhibit sporulation. This phenotype is conserved among *C. difficile* strains VPI 10463, CD630 and R20291. Co-culture in a trans-well system and on solid medium suggests that inhibition of sporulation is contact-dependent. Finally, cytological profiling using fluorescence microscopy suggests that enterococci inhibit the initial decision to enter sporulation before the construction of the asymmetric division septum. Taken together, these data suggest that enterococci may deplete metabolites necessary for entry into sporulation. Manipulation of such metabolites may be useful in the development of decolonization treatments for asymptomatically colonized individuals.

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GENOMIC SURVEILLANCE FOR PROOFREADING DYSFUNCTION IN THE *CLOSTRIDIOIDES DIFFICILE* POLYMERASE AND HISTIDINOL PHOSPHATASE (PHP) DOMAIN OF THE POLC-TYPE DNA POLYMERASE III ALPHA-SUBUNIT (POLC)

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Background: DNA replication proofreading in *Clostridioides difficile* is maintained by the polymerase and histidinol phosphatase (PHP) domain of the PolC-type DNA polymerase III alpha-subunit (PolC). Prior studies revealed the loss of one metal-coordinating residue of the PHP creates a '*hypermutator*' phenotype with reduced proofreading and 3' – 5' exonuclease function. The purpose of this study was to explore the conservation of the nine metal-coordinating residues in the PHP domain of PolC between *C. difficile* and the Bacillota phylum.

Methods: This study used CLC Genomics (Qiagen) for analyzing a custom microbial database of 979 *C. difficile* PolC sequences and 824 non-redundant, representative Bacillota phylum PolC sequences. Protein structure prediction was performed in AlphaFold2 (DeepMind) via ColabFold and modeling using Maestro (Schrodinger) and UCSF ChimeraX.

Results: We compared the known nine metal-coordinating residues of the *Bacillus subtilis* PolC (BsuPolCH338, H340, D347, H372, E397, H613, C663, N736, and H738) to those relative positions of *C. difficile* and the *Bacillota phylum*. We find that eight of nine metal-coordination PHP positions are highly conserved (>90%) across the phylum, but one position (BsuPolCN736; CdiPolCD731) appears uniquely dimorphic (~50% asparagine, ~50% aspartate). Further analysis of 979 *C. difficile* PolC sequences reveals 100% conservation at all nine positions.

Conclusion: PHP-mediated DNA replication proofreading appears to be highly conserved in *C. difficile*. Interestingly, we found a unique phylum-level dimorphism at one position that mediates manganese2+ coordination of the PHP domain of PolC. Surveillance for PolC PHP proofreading dysfunction is a potential new way to monitor runaway evolution due to a '*hypermutator*' phenotype.

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TRANSIENT RECEPTOR POTENTIAL VANILLOID 4 (TRPV4) REGULATES INFLAMMATORY RESPONSE AND CELL DEATH INDUCED BY *CLOSTRIDIOIDES DIFFICILE* TOXINS A AND B IN ENTERIC GLIAL CELLS

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The transient receptor potential vanilloid 4 (TRPV4), a non-selective cation channel, participates in the pathogenesis of intestinal bowel disease (IBD), which is a risk factor for severe *Clostridioides difficile* infection (CDI). Enteric glia, which is part of the enteric nervous system (ENS), is dramatically affected by IBD and *C. difficile* toxins. Here, we evaluated whether enteric glia expresses TRPV4 under physiological conditions and during CDI, as well as we investigated the role of this receptor in the inflammatory response and cell death induced by *C. difficile* in enteric glia. Mice were infected by *C. difficile* and euthanized on day 3 post-infection (pi) to evaluate TRPV4 expression in the cecum and colon by immunohistochemistry. *In vitro*, enteric gliawere incubated with TcdA (50ng/mL) or TcdB (1ng/mL) for 18h in the presence or absence of the TRPV4 antagonist (RN-1734, 100 μM). Then, cells were collected to analyze TRPV4 expression qPCR, Western blotting and immunofluorescence), inflammatory response (NFκB, TNF-α, IL-6 and IL-1β) and cell death (phosphatidylserine-annexin V binding, cleaved caspase-3 and *bcl*-2 expression). Increased TRPV4 was detected in the epithelium cells, submucosa and myenteric plexus cells in infected mice compared to uninfected. *In vitro*, TcdA and TcdB increased the TRPV4 expression on enteric glia. Blockage of TRPV4 resulted in decreased NFκB nuclear translocation followed by reduced levels of TNF-α and IL-6 in enteric glia challenged with TcdA, but not TcdB. On the other hand, TRPV4 antagonist decreased *C. difficile* toxins-induced enteric glia apoptosis by reducing the activation of caspase-3 and by upregulating the antiapopototic factor *bcl*-2. Based on these findings, we conclude that TRPV4, which is upregulated during CDI, plays an important role on the deleterious effects of *C. difficile* toxins on enteric glia by intensifying the inflammatory response and inducing cell death via activation of caspase-3 and downregulation of antiapoptotic mediator.

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CLOSTRIDIOIDES DIFFICILE INTERACTION WITH THE COMPLEMENT SYSTEM: IMPLICATIONS IN PATHOGENESIS

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Clostridioides difficile infection (CDI) is the most common cause of healthcare-associated diarrhea in the United States. It is well documented that risk factors for primary and recurrent CDI include a gut microbiota in dysbiosis and absence of protective immunity. It is unclear how *C. difficile* interacts with innate immunity to shape disease progression and protective immunity. The complement is part of the innate immune defense against microbes. Even though complement is mainly derived from hepatocytes and considered a serum-based host defense system, gut complement has recently been shown to be active in the gut. Hence, in this work, we studied the interaction of complement with *C. difficile* and identify the impact on CDI. Our results show that *C. difficile* spores interact with complement molecules C1q and C3 in a dose-dependent and antibody-independent manner. Additionally, *C. difficile* spores promote C3 degradation in serum, indicating that the spore alone can activate complement. Using far-western blotting, we identified that C1q and C3 bind to two components of the exosporium layer of *C. difficile* spores. Notably, using an ileal loop mouse model, we observed that *C. difficile* spores interact with gut complement molecules, C1q and C3. In an attempt to observe the protective role of complement against *C. difficile* in a mice model of infection with C1q knock out mice. Strikingly, we observed an opposite effect, that lack C1q KO mice had faster recovery from primary CDI and developed milder diarrhea during relapse of CDI compared to wild-type mice. Our in progress work seeks to confirm the interacting proteins of *C. difficile* spores and vegetative cells with complement molecules and how C1q impacts disease progression. Collectively, these results provide evidence that complement is modulated by *C. difficile* and that at least the classical pathway (C1q) is impacts recovery and recurrent CDI.

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ATOMIC STRUCTURE OF DIFFOCIN, AN R-TYPE BACTERIOCIN OF *CLOSTRIDOIDES DIFFICILE*

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Most *C. difficile* isolates encode an R-type bacteriocin, diffocin, which possibly functions as a defense against competing strains. Because of the extremely high bactericidal potency of these contractile nanotubes, they have been explored as antimicrobial agents for prevention and treatment of CDI, and Pylum Biosciences has engineered these particles to have specificity against strains of clinical significance. We elucidated the atomic structure of diffocins in the contracted and uncontracted forms, the first for any such structure for gram positives. Compared to the pyocins of *P. aeruginosa*, diffocins have a much shorter degree of contraction and the tube likely does not penetrate the entire cell wall. Prominent on the baseplate structure is a hydrolase domain facing directly downward and in contact with the cell surface, we hypothesize that this domain is crucial in degrading penetrating the cell wall and/or S-layer structure unique to *C. difficile*. This work is also the first to resolve the tape measure protein, housed as a trimer in the inner tube, which are injected into the inner membrane and likely plays a role in cell death.

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GENETIC DISSECTION OF THE ROLE OF SELENOPROTEINS IN *CLOSTRIDIOIDES DIFFICILE* PHYSIOLOGY

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The specific incorporation of selenium into macromolecules, both protein and nucleic acids, has been studied for more than five decades. Many of the microbes that use selenium in key systems for bioenergetics are strict anaerobes that rely on metalloenzymes to couple redox reactions to balance catabolic and anabolic pathways to thrive without potent electron sinks like oxygen. *Clostridioides difficile* has emerged as a genetic model system to dissect the pathways for specific insertion of selenium into both selenocysteine-containing proteins as well as labile selenoenzymes. One group of labile selenoenzymes is the selenium-dependent molybdenum hydroxylases (SDMH) which possess a unique version of selenium in the form of an uncharacterized labile cofactor; however, the insertion and maturation of the cofactor has never been genetically characterized. These enzymes are likely the first dedicated steps for catabolism of purines that are abundant in nature as vital nitrogen and carbon sources for anaerobes.

PURPOSE: In this study, we use a recently developed CRISPR-Cas9 system to probe the required gene products for maturation of the labile selenium cofactor in SDMH enzymes in *C. difficile.*

METHODS and RESULTS: We first observed that *C. difficile* could utilize hypoxanthine (HX), xanthine (X) and uric acid (UA) for growth in a minimal medium, but only when glycine and threonine were omi>ed. In this specific medium, we found that strains unable to make selenoproteins (*selD* mutants) were unable to use X and UA. Previous computational studies have identified *smhA* and *smhB* as potential candidates for cofactor maturation, so we subsequently deleted each gene using CRISPR-Cas9 technology. We surprisingly found that the ΔsmhA mutant mimicked the *selD* phenotype while the ΔsmhB mutant displayed no obvious change in growth.

CONCLUSIONS: Our studies ultimately provide the first genetic evidence for Se-dependent purine catabolism while also showcasing *C. difficile* as an appropriate model organism to study the biological use of selenium.

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INCREASED *IN VITRO* INTESTINAL ADHERENCE OF REA GROUP Y (RT014/020) COMPARED TO REA GROUP BI (RT027)

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Despite marked changes in the molecular epidemiology of other *Clostridioides difficile* strains over the past 40 years, restriction endonuclease analysis (REA) group Y (PCR ribotype [RT] 014/020) has remained prevalent world-wide. Previous data have demonstrated that the S-layer gene cassette plays a critical role in *C. difficile* epithelial cell adhesion, which is a required for *C. difficile* intestinal colonization.

We measured *in vitro* intestinal adherence of 19 *C. difficile* isolates, including 12 isolates identified as REA group Y (RT014/020), 6 isolates identified as REA group BI (RT027), and 1 isolate identified as REA group BK (RT078/126). Whole genome sequencing was performed on all isolates and the S-layer cassette types (SLCT) were analyzed.

REA group Y isolates had a notably greater *in vitro* intestinal adherence as 22.4% (95% CI: 17.1% - 27.7%) adhered to Caco-2 cells compared to only 5.5% (95% CI: 3.7% - 7.3%) of REA group BI isolates. ($p<0.001$) Groups Y and BK (22.6%) had comparable *in vitro* intestinal adherence. Group Y isolates had two distinct SLCTs (Types 6 and 10) with the most variation noted within the genes for surface-layer protein A (*slpA*). Additionally, group Y isolates had two variations of cell wall protein 84 (*cwp84*), but there was a 99.4% sequence homology between these two variants. Conversely to group Y isolates, REA group BI isolates had only one SLCT (Type 4) with minimal variation within genes that make up the S-Layer gene cassette.

Differences between the S-layer gene cassettes of REA group Y and BI isolates correlated with a significant difference in *in vitro* epithelial cell adhesion. These differences likely provide a competitive advantage for colonization by REA group Y and a potential explanation for long term persistence of this strain group.

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CLOSTRIDIOIDES DIFFICILE TOXIN-MEDIATED CONTROL OF BILE ACID PRODUCTION VIA FARNESOID X RECEPTOR ACTIVATION

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C. difficile remains an urgent priority, and novel non-antibiotic treatments are needed. The pathogen's life cycle is exquisitely sensitive to alterations in the gut microbiota-mediated bile acid pool. The master regulator of bile acid production in the host is the Farnesoid X Receptor, FXR. FXR activation also reduces inflammation by inhibiting pro-inflammatory cytokine expression. In successful FMT for recurrent CDI, FXR levels are elevated, although this was not seen in patients with primary CDI. Thus, FXR activation may provide a new target to reduce CDI severity however, the relationship between CDI and FXR is not well understood. Monolayers of Caco-2 cells and primary colonocytes from human donors were exposed to *C. difficile* toxins, TcdA, TcdB, or both at concentrations ranging from 10-100 pM for 24 hr. RNA was extracted for RNAseq (primary cells) or qRT-PCR (Caco-2 cells) to look for changes FXR-genes. Tissue from WT C57BL6J mice (n=4 per group) and antibiotic treated mice with and without CDI were used to define liver, ileal and cecal tissue gene expression profile using the Nanostrings Human Host Response panel with custom FXR pathway genes. FXR expression significantly increased in both TcdA (30 pM) and TcdB (30 pM) treated primary colonocytes and Caco-2 cells. Significant increases in FXR were observed in the liver, but not the cecum, of mice with CDI compared to antibiotic treated only mice. In the liver *Cyp7a1*, the rate limiting enzyme in bile acid synthesis, had significant decreases in expression, indicating a reduction in bile acid production with CDI mediated by FXR. In the cecum, other nuclear receptors were altered as well, including PXR, VDR, CAR, and PPARα. These data shed light on a new potential mechanism of how *C. difficile* toxins are able to control host bile acid synthesis via FXR regulation. Exploring the relationship between CDI, FXR and host response may help identify alternate host targets for treating CDI.

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REVEALING ROLES OF S-LAYER PROTEIN (SLPA) IN *CLOSTRIDIOIDES DIFFICILE* PATHOGENICITY BY GENERATING THE FIRST *SLPA* GENE KNOCKOUT MUTANT

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Clostridioides difficile infection (CDI) with high morbidity and high mortality, is an urgent threat to public health, and *C. difficile* pathogenesis studies are eagerly required for CDI therapy. The major S-layer protein, SlpA, was supposed to play a key role in *C. difficile* pathogenesis, however, a lack of isogenic *slpA* mutants has greatly hampered analysis of SlpA functions. In this study, a CRISPR-Cas9 system was constructed, and based on this system, the whole *slpA* gene was successfully knocked out for the first time. Deletion of *slpA* in *C. difficile* resulted in smaller, smother-edged colonies, shorter bacterial cell size, and aggregation in suspension. For life cycle, the mutant demonstrated lower growth (OD600 changes) but higher cell density (colony numbers, CFU), decreased toxins production, and inhibited sporulation. Morevoer, the mutant was more impaired in motility, more sensitive to vancomycin and Triton-induced autolysis, releasing more lactate dehydrogenase. In addition, SlpA deficiency led to robust biofilm formation but weak adhesion to human host cells. Results in this study will provide direct proof for roles of SlpA in *C. difficile* pathogenesis, which will facilitate future investigations for new targets as vaccines, new therapeutic agents, and intervention strategies in combating CDI.

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