ANAEROBE CONGRESS
JULY 7-10, 2026

COLUMBIA UNIVERSITY IRVING MEDICAL CENTER
Roy and Diana Vagelos Education Center
104 Haven Avenue (at W. 171st Street)
New York, NY 10032

ABSTRACT BOOK

Anaerobe 2026

July 7-10, 2026

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Dear Colleagues and Friends,

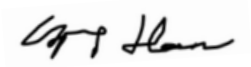
It is my great pleasure and honor to welcome you to the 18th Biennial Anaerobe Congress of the **Anaerobe Society of the Americas (ASA)**, held at the beautiful Vagelos Education Center on the campus of Columbia University Irving Medical Center.

This Congress brings together investigators, clinicians, educators, trainees, and industry partners from around the world who share a commitment to advancing our understanding of anaerobic bacteria and the diseases they cause. Over the next three days, you will hear presentations showcasing advances in basic science, clinical microbiology, diagnostics, antimicrobial resistance, host-microbe interactions, the microbiome, and emerging therapeutic approaches. Just as importantly, this meeting offers opportunities to exchange ideas, build collaborations, mentor future leaders, and strengthen the professional connections that define our Society.

I sincerely thank our speakers, poster presenters, session chairs, patrons, exhibitors, judges, volunteers, and members of the **ASA** Board, who also served as the Organizing Committee. Your dedication and generosity made this Congress possible. We are honored to welcome keynote speaker Dr. Vincent Young, a distinguished leader in anaerobe bacteriology and microbiome research. At Friday's Awards Banquet, we will celebrate Dr. Stuart Johnson as the recipient of **ASA** Lifetime Achievement Award and honor the memory of Dr. Mike Cox, a founding member of **ASA** whose enduring legacy has inspired generations of scientists. I also extend my heartfelt thanks to former ASA Executive Director Dr. Ron Goldman, Immediate Past President Dr. Vincent Young, and my colleagues on the **ASA** Board for their guidance and support throughout the planning of this meeting.

Thank you for joining us and for your continued contributions to our field. I wish you a stimulating, productive, and enjoyable Congress, and I hope you have the opportunity to experience all that New York City has to offer.

With my warmest welcome,



Yiping Han, PhD
President and Congress Chair
Anaerobe Society of the Americas

About the Anaerobe Society

Founded in 1992, the **Anaerobe Society of the Americas,** is a non-profit foundation that serves as a forum for those interested in anaerobic bacteriology, anaerobic infections, and related matters. The Society aims to:

1. Stimulate interests in anaerobic biology and to encourage interaction and collaboration among researchers and clinicians from all disciplines, including medical, dental, veterinary, environmental, and basic sciences;
2. Bring together researchers and clinicians interested in anaerobic biology and anaerobic infection for formal and informal meetings, including the signature biannual congress;
3. Review and assess new advances in the field;
4. Discuss areas of controversy; and
5. Identify 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 18th biennial Anaerobe Society Congress. Past **Anaerobe Society of the Americas** sponsored programs include:

ANAEROBE 2024—Ann Arbor, MI USA
ANAEROBE 2022—Seattle, WA USA
ANAEROBE 2020—Virtual
ANAEROBE 2018—Las Vegas, NV USA
ANAEROBE 2016—Nashville, TN USA
ANAEROBE 2014—Chicago, IL USA
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ANAEROBE 2010—Philadelphia, PA USA
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ANAEROBE 2000—Manchester, England
ANAEROBE 1998—Buenas Aires, Argentina
ANAEROBE 1996—Chicago, IL USA
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ANAEROBE 1992—Los Angeles, CA USA

Anaerobe Congress 2026

The 18th biennial Congress of the **Anaerobe Society of the Americas** will address both the clinical and microbiological aspects of anaerobes, as related to human diseases, animal diseases, and environmental conditions. Mixing theory and practical applications, the Congress will consist of invited speakers, oral presentations, and poster presentations, representing work by researchers from many different countries.

Curricular Goals & Objectives

This year's congress aims to:

- Provide information on the latest developments in research on anaerobic microorganisms, including the role of anaerobes in disease, the epidemiology of anaerobic infections, potential prevention strategies, and research of anaerobes in the environment.
- 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 anaerobic microbiology labs for delivery of patient care.

Accreditation & Certificates of Attendance

ANAEROBE 2026 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 the 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

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.

Patrons & Exhibitors

The **Anaerobe Society of the Americas** gratefully acknowledges the following organizations for their generous support of the 18th Biennial Congress.

Support for this activity was received in donations from:

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- Anaerobe Journal
- ASM Journals (Sponsor of Mike Cox Student Poster Award)
- Stellar Services

The following patrons are tabling at Anaerobe 2026:

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Keynote Speaker

Vincent Young, MD, PhD

Dr. Young is the William Henry Fitzbutler Collegiate Professor in the Department of Internal Medicine/Infectious Diseases Division and the Department of Microbiology & Immunology at the University of Michigan Medical School. He received his undergraduate degree from the Massachusetts Institute of Technology in 1985 and received his M.D. and his Ph.D. in Microbiology & Immunology from Stanford University 1992 under the joint guidance of Gary Schoolnik and Stanley Falkow. He completed his clinical training in Internal Medicine and Infectious Diseases at the Massachusetts General Hospital followed by postdoctoral studies at MIT. He was previously on the faculty at Michigan State University prior to joining the University of Michigan in 2007.

Dr. Young has a long-standing interest in understanding the pathogenesis of bacterial infections of the gastrointestinal tract and the role of the normal microbiota in human health and disease. As part of the NIH Human Microbiome Project, Dr. Young led a team that studied the role of the microbiome in inflammatory bowel disease. Current research in the Young Lab includes a team science effort to understand the pathogenesis of infection with *Clostridioides difficile* and antimicrobial-resistant bacterial pathogens. He is leading a team using an integrated approach that combines clinical research, bacterial genomics, microbial ecology and immunology/host response projects. He is also studying how the microbiota changes with age and how this influences the health of aging individuals.

A key aspect of Dr. Young's research is translating basic research to clinical practice and also observing trends in clinical care to drive the research done in the laboratory. The lab's focus on healthcare-associated infections is helping define the unintended consequences of modern healthcare related to the rise of antibiotic resistance and disruption of the microbiome. Through a detailed understanding of the interactions between the indigenous microbiota, pathogens and the human host, the Young Lab hopes to improve our ability to treat infection while minimizing long-term adverse consequences to human health.

In Memoriam

Mike Cox

The Anaerobe Society of the Americas honors the contributions of **Mike Cox (1945-2024)** to **ASA** and the field of anaerobic bacteriology. He dedicated his life to improving methods for the study of anaerobic bacteria and educating clinical microbiologists and researchers on anaerobic bacteriology. His career spanned over 50 years and impacted many scientists in the field. Among the preeminent experts in anaerobic microbiology, he founded Anaerobe Systems in 1978 and pioneered laboratory technology to more easily manipulate and work up anaerobic organisms under completely anaerobic conditions, including inventing and patenting the first gloveless anaerobic chamber. He continued technical innovations with designing processes to make renewable hydrogen by the anaerobic fermentation of agricultural waste.

He was a founding board member of the **ASA** and helped to financially support the organization. He conducted workshops at the Anaerobe Congresses for over 20 years and supported anaerobic studies at such schools as Santa Clara University. He served on numerous industry subcommittees on anaerobic identification and susceptibility testing and co-authored the CLSI M56-A guideline "Principles
and Procedures for Detection of Anaerobes in Clinical Specimens". He was awarded the Anaerobe Society's Lifetime Achievement Award in 2014. He was a good friend to many microbiologists at **ASA** and around the world and always had a seat at the table for a new scientist. His numerous trainees, including his children, as well as his technical contributions that make anaerobes easier to culture, continue his legacy in anaerobic bacteriology.

In honor of Dr. Cox's memory, the student poster awards will be named "**Mike Cox Poster Award**" starting at Anaerobe Congress 2026

Lifetime Achievement Award

Stuart Johnson, MD, FIDSA, DTM&H

The **Anaerobe Society of the Americas** awards its 2026 Lifetime Achievement Award to Stuart Johnson, M.D, FIDSA, DTM&H at the Anaerobe 2026 Congress, being held July 7-10 at Columbia University Irving Medical Center in New York. This biennial award recognizes career accomplishments to the field of anaerobic bacteriology.

Dr. Johnson is an Infectious Disease Researcher at the Hines VA Hospital, Hines IL and an Emeritus Professor of Medicine at Loyola University Stritch School of Medicine Chicago. Dr. Johnson received his MD degree from the University of Minnesota Medical School and completed his internal medicine residency and infectious disease fellowship training at the University of Minnesota Hospital and the Minneapolis VA Medical Center. In 1993, he was recruited to Chicago by his mentor, Dale Gerding, M.D. and was on the faculty of the Lakeside VA Hospital and Northwestern University. He subsequently was recruited to the Hines VA Hospital and Loyola University. He received a Diploma in Tropical Medicine and Hygiene at Mahidol University in Bangkok, Thailand and a Career Development Award from the Department of Veterans Affairs.

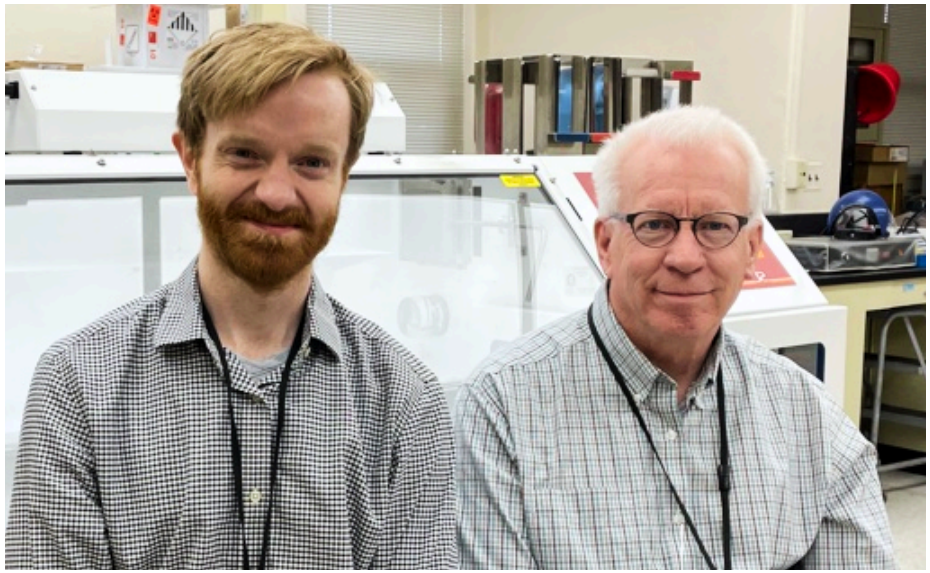
His main research interest and focus over the last 40 years has involved the epidemiology, pathogenesis, and treatment of Clostridioides difficile infection (CDI). His work has highlighted the risk of specific antibiotic resistant C. difficile strains to cause outbreaks in institutions that have high usage patterns of those antibiotics, the role of non-toxigenic C. difficile strains, and the importance of toxin B in the virulence of C. difficile.

His clinical experience in the treatment of patients with recurrent CDI has translated into studies helping to define optimal management of these infections. He is currently the Co-Principal Investigator of a multi-center, randomized, controlled trial within the VA Healthcare system designed to define optimal antibiotic management of early recurrences of CDI (results of which are soon to be published in New England Journal of Medicine Evidence).

(CONT.)

He was previously the Chair of the IDSA/SHEA (Infectious Diseases Society of America/Society for Healthcare Epidemiology of America) CDI Guidelines Committee for a focused update on management of CDI. He recently received the Lifetime Achievement Award from VASPID (the VA Society for Practitioners of Infectious Disease). He is a past President of the **Anaerobe Society of the Americas** (2006-2008).

In addition to his CDI research, he has been involved in clinical research on the parasite, Angiostrongylus cantonensis, responsible for most cases of eosinophilic meningitis, world-wide.



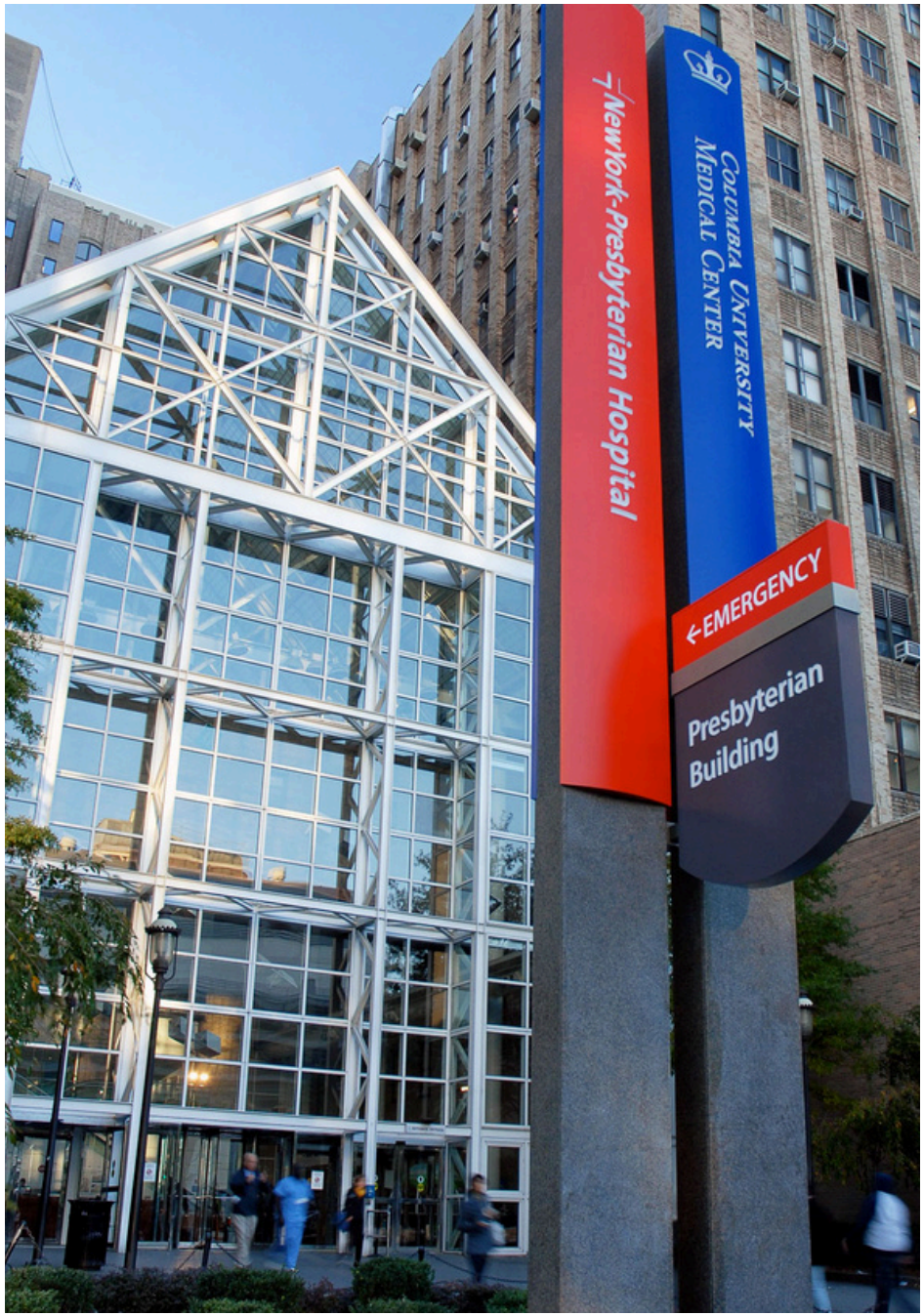
Disclosure Information

This Congress has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Med ical Education (ACCME). The **Anaerobe Society of the Americas** has attempted to ensure balance, independence, objectivity, and scientific rigor in this continuing medical education activity. All individuals in a position to con trol 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 in each presentation.



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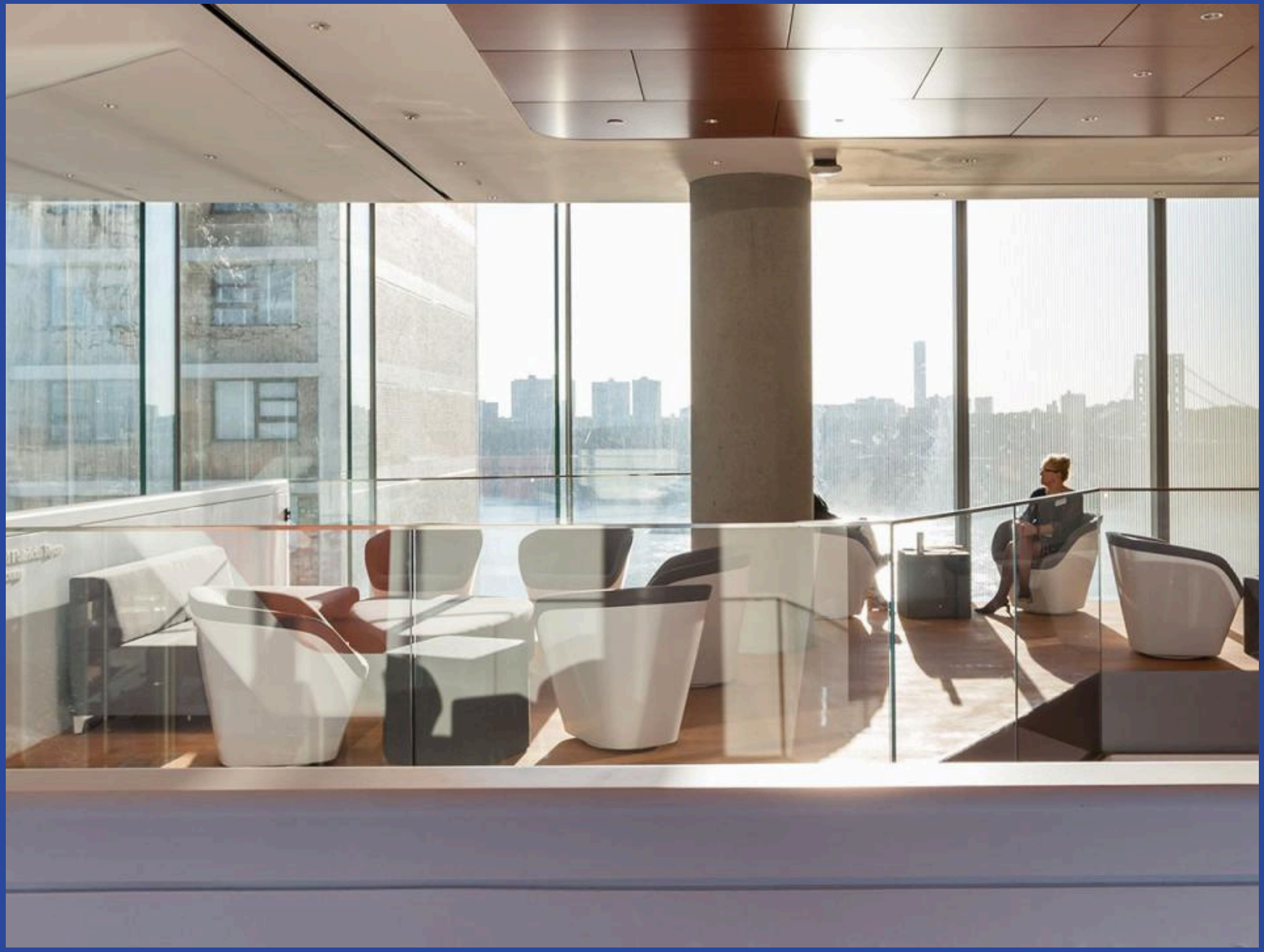

Oral Abstracts

This abstract book is divided according to the congress sessions listed in the program. The table below identifies the pages pertaining to each **oral presentation** session.

Wednesday, July 8

[I] Congress Keynote

*Indicates presenter

Microbe-microbe Interactions in the Gut: Systems Biology of Host-Microbe Symbiosis

Young V.[1]
1. Department of Internal Medicine/Infectious Diseases and the Department of Microbiology & Immunology at the University of Michigan Medical School, Michigan, MI, USA

The gastrointestinal (GI) tract of mammals plays host to complex communities of microbes. Amongst the bacterial inhabitants of the GI tract, most of which are obligate or facultative anaerobes. Under normal circumstances, the bacterial communities exhibit a stable community structure, which is in turn thought to form the basis for a stable symbiosis with their host. In turn, changes in the status of the indigenous microbiota or the host can disrupt GI homeostasis and have negative consequences for each participant in this symbiosis. We have been studying the nature of host-microbe symbiosis in several systems. Clostridioides difficile infection (CDI) is the exemplar of a disease that is reflective of the complex interactions between the pathogen, host and the indigenous microbiota. Furthermore, as a hospital-acquired infection, the epidemiology of the pathogen is critically important in developing strategies to limit the burden of disease in the population. CDI following the administration of antibiotics has been estimated to be the costliest healthcare-associated infection. In the US, CDI is responsible for an estimated 5 billion dollars in increased healthcare cost annually due to an estimated 500,000 cases and up to 29,000 deaths. Our previous work and the work of others have demonstrated that susceptibility to CDI and subsequent outcome of infection is driven by the myriad interactions between the host, microbiota and pathogen, all in the context of the healthcare environment. This complex interplay lends itself to study via a translational, systems biology approach. I will provide an overview of the studies of CDI in terms of this multiscale research approach. In addition, I will discuss recent work that has followed how the indigenous microbiota changes over the lifespan in experimental murine systems. I will present data that suggests that changes in both the microbiota and the host associated with aging can be correlated with differential susceptibility to disease including CDI.

Presented by
VINCENT B. YOUNG

Contact
YOUNGVI@MED.UMICH.EDU

Wednesday, July 8

[II] Anaerobes: Clinical Infections

*Indicates presenter

The Spread of *C. difficile* and Other Gut Pathogens Within the Healthcare Environment

Freedberg D.[1]
1. Columbia University, New York City, NY USA

Attempts to trace the spread of *C. difficile* within the healthcare environment have been surprisingly unsuccessful. When *C. difficile* is characterized genetically, a shockingly small proportion of *C. difficile* infections can be traced back to other patients who had *C. difficile* infection. This clinical-epidemiological talk will review the changing epidemiology of *C. difficile* during the last hundred years. Understanding *C. difficile* as a phased epidemic which is currently in the deceleration phase will inform understanding of how *C. difficile* does--and does not--spread within the healthcare environment. The talk will discourage the common perception that *C. difficile* (and other bacterial pathogens with strong tropism for the gut) are hospital-acquired. Rather, most of these pathogens are acquired in the community and spread from the patient into the hospital rather than vice-versa.

Presented by **DANIEL FREEDBERG**
Contact **DEF2004@CUMC.COLUMBIA.EDU**

Campylobacter-Associated Alterations of the Gut Microbiota in Young Children in Rural Ethiopia

Joosens M.[1]
1. University of Michigan Medical School, Michigan, MI USA

Campylobacter is the leading cause of bacterial childhood diarrhea and gut microbiota are suggested to influence infection susceptibility. Data on *Campylobacter*-associated gut microbiota profiles is however scares. We therefore collected stool samples from 296 children with diarrhea in an area of Ethiopia with a high burden of *Campylobacter* infections. All samples were examined for the presence of *Campylobacter* and the impact on the microbiome was assessed.

Campylobacter was identified with complementary methods and was found to be highly prevalent in the cohort (n=123; 42%), particularly in young children aged 6-11 months (prevalence= 68%). Gut microbiome profiling revealed that *Campylobacter* infection was associated with differences beyond the effects of the major confounding factors, age, and diarrhea. Overall, *Campylobacter*-infected children presented with less-developed gut bacterial communities across all age categories compared to their uninfected counterparts and a higher prevalence of bacteria typically associated with the oral microbiome.

We found *Campylobacter* infection to be associated with reduced gut microbiota richness and delayed maturation, independent of diarrhea and age, corroborating a role for microbiota immaturity in childhood infection risk.

Presented by **MARIE JOOSSENS**
Contact **MARIE.JOOSSENS@UGENT.BE**

Incidence and antimicrobial resistance of *Fusobacterium* spp. bloodstream infections in Sweden from 2015-2024

Nygren D.[1*] , Aspevall O.[2] , Torrison G.[1]
1. Department of Infectious Diseases, Skåne University Hospital, Malmö, Sweden.
2. The Public Health Agency of Sweden, Stockholm, Sweden

Background: Invasive disease due to *Fusobacterium necrophorum* has increased, potentially due to changing antibiotic prescription patterns for throat infections. Systematic investigations of other *Fusobacterium* spp. infections are rare, although an increase of *F. nucleatum* in Japan has been reported, but not seen elsewhere (e.g. in Italy or Australia). This study was designed with the aim to investigate incidence of bacteremia with *Fusobacterium* spp.

Methods: This was an observational registry-based study from 2016-24 using data from the Swedish Antimicrobial Resistance Surveillance Network (Svebar), to which participating microbiology laboratories in Sweden report culture findings. Case eligibility was a blood culture with a finding of *Fusobacterium* spp. Findings were categorized as *F. necrophorum*, *F. nucleatum* and *F. species*. Given anonymization of data, any two laboratory findings which shared the same species, region, age, sex and date, was considered a duplicate and were deduplicated. Yearly incidence rates were calculated. A logaritmic linear regression model was fitted and average annual percentage change (AAPC) described, with 95% binomial confidence intervals (95CI) provided.

Results: In total, 1352 episodes of *Fusobacterium* spp. bacteremia were recorded during 80 041 449 person years. Overall incidence was 4.7 (95CI 4.0-5.3) per million people per year for *F. necrophorum* (n=374), 5.8 (95CI 4.6-6.9) for *F. nucleatum* (n=475) and 6.1 (95CI 4.7-7.5) for *F. species* (n=503). From 2016-24, the AAPC was 1% (95CI -4-6%) for *F. necrophorum*, 9% (95CI 4-14%) for *F. nucleatum* and 7% (95CI -0.5-15%) for *F. species*.

Most isolates included in the F. species group were not determined to the species level (91%), Those that were, consisted of F. gonidiaformans (n=15), F. mortiferum (n=7), F. naviforme (n=7), F. nucleatum/naviforme (n=5), F. periodonticum (n=5), F. varium (n=5) and F. canifelinum (n=1).

Presented by **DAVID NYGREN**

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(CONT.)

Conclusions: Among *Fusobacterium* spp. only *F. nucleatum* bacteremia increased in Sweden during 2016-24. *F. necrophorum* bacteremia did not increase during the study period, but the incidence rate was at the highest level yet described.

Wednesday, July 8

[III] Anaerobes: Disease Mechanisms

*Indicates presenter

Using Genetics to Explore Microbe-Host Interactions at the Molecular Level

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The human gut microbiota is occupied by anaerobic Clostridia that remain difficult to genetically manipulate, limiting mechanistic studies of microbiome function. We developed a broadly applicable genetic toolkit for diverse non-model gut Clostridia, including optimized promoters, inducible gene expression systems, and compact CRISPR-based genome editing platforms. These tools enable efficient and precise genetic manipulation across multiple gut commensal species. As a proof of concept, we engineered TMA-metabolizing bacteria and achieved inducible control of metabolite production in vivo. This platform expands the genetic accessibility of dominant gut microbes and provides a foundation for mechanistic microbiome research and next-generation live bacterial therapeutics.

Presented by
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Predicted Histidine Kinase Functions as a Novel Serine-Aspartate Kinase/Phosphatase to Directly Control *C. difficile* Sporulation

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The anaerobe, *Clostridioides difficile*, relies on the formation of dormant spores for survival outside of the mammalian gastrointestinal tract. The differentiation of the vegetative cell to the spore form is directed by Spo0A, the master transcriptional regulator of sporulation. Spo0A is activated by phosphorylation in all spore-forming bacteria; however, the factors that mediate *C. difficile* Spo0A phosphorylation are not conserved nor are well understood. Two proteins are known to interact with Spo0A: the predicted kinase, PtpC, and the small Spo0A inhibitor, Spo0E. PtpC is a phosphotransfer protein that was previously shown to interact and phosphorylate Spo0A. However, a *ptpC* mutant hypersporulates, suggesting it negatively regulates Spo0A. We present evidence that suggests Spo0E facilitates the interaction of PtpC with Spo0A *in vivo*. Through *in vivo* and *in vitro* experiments, we also demonstrate that PtpC acts as both a kinase and a phosphatase on Spo0A. But, despite PtpC containing a canonical HisKA motif and being annotated as a histidine kinase, we found that the conserved histidine residue was not involved in phosphotransfer with Spo0A. Surprisingly, PtpC kinase and phosphatase activities require serine residues within the C-terminus catalytic domain, which are predicted to interface with the conserved aspartate of Spo0A. The PtpC serine-aspartate kinase and phosphatase activity is the first of its kind identified and represents a novel mechanism for the regulation of sporulation.

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A Targeted Cell Lysis Mechanism Facilitates Toxin Release in *C. difficile*

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Clostridioides difficile infection is associated with the production of two large toxins, TcdA and TcdB. The toxins are encoded in a pathogenicity locus along with a phage-like holin protein, TcdE, the remnants of a phage endolysin, TcdL, and a positive transcriptional regulator, TcdR. Previous studies investigating the mechanism of toxin secretion from the bacterium have yielded disparate results as to whether the bacteria undergo lysis and whether TcdE is required. Here, we demonstrate that TcdE, TcdA, and TcdB are only expressed in a small subset of cells in culture. The cells with high TcdE and toxin expression show disrupted membranes, consistent with a form of bacterial lysis. The artificial overexpression of TcdE, TcdA, and TcdB through TcdR induction promotes *tcdE*-dependent bacterial lysis and cell death, even in 630*Δerm*, a strain previously thought to have tcdE-independent toxin secretion. We propose a general mechanism where a minority subpopulation undergoes TcdE-mediated lysis to release toxins, reconciling studies with differences stemming from strain variability and population-level assays.

Presented by

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Wednesday, July 8

[IV] Host Responses to Anaerobic Infections

*Indicates presenter

Micriobiota-Generated Metabolites and Impact on Host Responses

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The goal of our research is to understand and control the chemistry of human-associated bacteria in order to uncover how the microbiome affects human health and disease. Our current work is focused on how human gut bacteria metabolize host-produced small molecules and how the resultant compounds affect host physiology, and in particular, host metabolism, immune function, and neurological function and behavior. Host-produced compounds such as bile acids, steroids, and sterols act as crucial signaling molecules and regulators of host biology, but their metabolism by gut bacteria has been relatively unexplored. Here I will discuss our recent progress toward uncovering the biosynthetic pathways and biological roles of bacterially modified metabolites, with an emphasis on how specific chemical transformations can alter host signaling and physiology. As one example, we have identified bacterial pathways that remodel host steroid scaffolds. This work reveals previously unappreciated microbial modulation of steroid chemistry and downstream host biology, with potential implications for women's health conditions including fertility, preeclampsia, and postpartum depression. As a second example, our recent work implicates bacterially modified bile acid metabolites as modulators of host intestinal circadian programs, highlighting a mechanistic link between gut microbial metabolism and host timekeeping pathways. These studies add to a growing body of evidence suggesting that processes that were once thought to be exclusively controlled by the host are in fact modulated by the gut microbiome.

Presented by
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Regulation of Early Life Immunity by the Gut Microbiota

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The early-life period is a critical window in which the gut microbiome orchestrates immune development and long-term tolerance. Our integrated work identifies complementary mechanisms spanning maternal-fetal and postnatal life. Maternal microbiota-derived tryptophan metabolites (indoles) expand MDSCs and RORγt$^+$ Tregs at the maternal-fetal interface, restraining pathogenic IFN-γ$^+$ and IL-17$^+$ T cells and preventing fetal loss. These pathways are conserved in humans and restored by microbial supplementation. Postnatally, maternal microbiota-induced IgG is transferred via breast milk and FcRn, calibrating the neonatal microbiome, limiting an enteric pathogen *Citrobacter rodentium* colonization, and preventing dysbiosis-driven pathogenic γδ T cells that exacerbate later colitis. Furthermore, we found that the neonatal gut is enriched with neurotransmitters; specific bacteria produce serotonin that directly promotes Treg differentiation via indole-3-acetaldehyde and mTOR inhibition, establishing durable tolerance to dietary antigens and commensals. Together, these layered maternal and neonatal microbial pathways reveal actionable targets for preventing immune-mediated diseases originating in early life.

Presented by
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Inflammation Fuels Metabolic Synergy Between Pathogens in the Oral Microbiome

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Periodontal disease is a prevalent chronic inflammatory condition that causes irreversible tissue destruction, tooth loss, and systemic comorbidities. Disease progression is driven by a self-perpetuating cycle in which inflammation selects for pathogenic microbiota that further amplify host immune activation. Current therapies broadly disrupt the oral microbiome, underscoring the need for targeted, microbiota-sparing approaches. However, the complexity of oral microbial communities has hindered the identification of actionable therapeutic targets. To address this challenge, we developed a simplified, disease-relevant model centered on the microbial dyad *Fusobacterium nucleatum* and *Campylobacter rectus*, two Gram-negative anaerobes that co-expand in early-stage periodontitis and are enriched in related comorbidities, including oral tumors. Using *in vitro* co-culture biofilms, bacterial mutants, transcriptomics, and *in vivo* models (murine subcutaneous abscess and ligature-induced periodontitis), we identified a cooperative metabolic interaction between these two species. *F. nucleatum* supports *C. rectus* growth by supplying formate as an electron donor, enabling *C. rectus* anaerobic respiration, while *C. rectus* reciprocally promotes the expansion of tumor-associated *F. nucleatum* subsp. animalis strains. *In vivo*, *C. rectus* is avirulent during mono-infection but expands markedly during co-infection with *F. nucleatum*, driving enhanced neutrophil recruitment and activation, including induction of inducible nitric oxide synthase (iNOS). Unexpectedly, rather than restricting bacterial growth, a major byproduct of iNOS activity – nitrate – serves as an electron acceptor that enables *C. rectus* to further exploit *F. nucleatum*-derived formate, amplifying *C. rectus* expansion and likely accelerating tissue destruction. Notably, tungstate selectively inhibits *C. rectus* anaerobic respiration without affecting F. nucleatum or commensal microbes, highlighting a potential precision therapeutic strategy. Together, these findings define an inflammation-driven metabolic network that sustains a pro-inflammatory niche and promotes periodontal disease progression.

Presented by
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Wednesday, July 8

[V] Anaerobes in the Oral Cavity

*Indicates presenter

The Ecological Effect of the Prebiotic Arginine on the Oral Microbiota

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Numerous studies have demonstrated that arginine can modulate the oral microbiome, restore pH homeostasis and reverse microbial dysbiosis. Arginine functions as a natural oral prebiotic that exerts a profound ecological effect on the oral microbiome by restoring microbial homeostasis rather than employing broad-spectrum biocidal action. By simultaneously boosting alkali production and disrupting the microbial matrix, arginine-based therapeutics can offer a promising new strategy for the long-term management of chronic oral diseases.

Presented by

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From Lone Microbe to Microbial Team: Understanding Anaerobic Partnerships Between *Veillonella* and *Prevotella*

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The human oral cavity harbors a highly diverse microbial ecosystem in which interspecies interactions shape community assembly, ecological stability, and disease susceptibility. Among the earliest obligate anaerobic colonizers of the oral microbiome, *Veillonella* and *Prevotella* species are frequently detected in both health and disease, yet the molecular basis of their interactions remains poorly understood. We have investigated the ecological and mechanistic interactions between *Veillonella* and *Prevotella* using microbial genetics, dual-species biofilm models, fluorescence imaging, transcriptomics, and in vivo colonization studies. We demonstrate that co-culture of *Veillonella parvula* with multiple *Prevotella* species, results in significant synergistic growth and enhanced biofilm formation compared with mono-species cultures. Fluorescence in situ hybridization revealed close spatial organization within dual-species biofilms, suggesting direct physical and metabolic cooperation. We further show that co-infection with *Prevotella* significantly enhances *Veillonella* colonization in vivo using a *Drosophila melanogaster* colonization model. Transcriptomic analyses identified major metabolic reprogramming in both organisms during co-culture, including upregulation of pathways involved in choline utilization, propanediol metabolism, and bacterial microcompartment formation, suggesting adaptive metabolic cross-feeding. Ongoing metabolomic and transposon mutagenesis studies aim to define the molecular determinants of this synergy. Our findings reveal a previously unrecognized cooperative interaction between *Veillonella* and *Prevotella* that may influence oral microbial homeostasis, inflammatory dysbiosis, and microbiome-associated systemic diseases.

Presented by
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Four's a Party: Intricate Interplay Between One Oral Bacterium, Its Two Parasites, and One Predatory Phage

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We utilized a multi-party microbial system to investigate the biological, ecological, and pathogenic effects of interactions among phylogenetically distant but ecologically intimate oral microorganisms.
A better understanding of oral microbial ecology and its impact on diseases calls for an appreciation of the multi-party interactions involving microorganisms of different categories, expanding from bacteria to include other under-investigated microbial types, such as bacteriophages and recently identified Candidate Phyla Radiation (CPR) organisms. Here, we describe a multi-party microbial system, comprising 1) an oral *Schaalia odontolytica* strain XH001; 2) a Saccharibacteria *Nanosynbacter lyticus* strain TM7x, which is the first culture representative of CPR bacterium with a highly reduced genome; 3) an XH001-encoded prophage xhp1, and 4) a lytic phage LC001 that targets XH001. TM7x lives on the surface of its host bacterium XH001, forming a unique epiparasitic relationship, which significantly affects the behavior of prophage xhp1, and modulates the interaction between XH001 and lytic phage LC001. Using comprehensive techniques and assays including bacterial genetics, Raman spectroscopy, imaging; bacteria-phage co-evolution and RNAseq, we demonstrated that: I) TM7x association triggers lipid droplet production in XH001, and this is mediated through XH001-encoded prophage xhp1, leading to enhanced survival of XH001 under stress conditions; II) The association of TM7x protects XH001 from infection by oral lytic phage LC001, promoting long-term coexistence of bacterium XH001 and its lytic phage within the oral cavity, which is of ecological significance; III) XH001-encoded prophage xhp1 is prone to activation when XH001 forms episymbiosis with TM7x. The released xhp1 induces antiviral immunity and inhibits antibacterial response in the mammalian host. Our study indicates that phages engage in intricate interaction with bacterial residents of the oral microbial community and exert a far-reaching influence on bacterial physiology, microbial ecology, and pathogenesis.

Presented by
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Thursday, July 9

[VI] Anaerobes in GI Health and Disease

*Indicates presenter

Metagenomic Editing of Commensal Bacteria in Situ Using CRISPR-Associated Transposases

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Culture-free metagenomic sequencing has revealed the extraordinary diversity of gut commensals that shape host nutrition, immunity, and health. Yet, few cultureindependent tools exist to directly manipulate these microbes within their native environment. To address these limitations, we developed **Metagenomic Editing (MetaEdit)**, a platform that delivers CRISPR-associated transposases into the gut microbiome to stably integrate kilobase-scale DNA payloads into targeted bacterial genomes in vivo. In murine models, MetaEdit enabled the "genetic capture" of a native *Bacteroides* strain by integrating an antibiotic resistance marker at high-specificity (>99% on-target across gigabases), facilitating its selective isolation from stool. We next reprogrammed this endogenous *Bacteroides* with an inulin-responsive operon, conferring diet-dependent growth control. Dietary inulin selectively expanded the engineered strain 30- to 40-fold over weeks, with rapid depletion upon diet withdrawal. Finally, we achieved the first direct genomic modification of Segmented Filamentous Bacteria (SFB), a potent immunomodulatory symbiont typically refractory to cultivation. Integration of a fluorescent reporter enabled visualization of edited filaments residing in the small intestine. Together, these findings establish MetaEdit as a paradigm-shifting technology for precise and durable editing of individual microbiome members, expanding the experimental toolkit for mechanistic discovery and paving the way toward therapeutic strategies that treat the microbiota itself as an editable, functional component of host biology.

Presented by

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Foundation Guild-The Stable Health-Relevant Group of Obligate Anaerobes in Human Gut Ecosystems

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The gut microbiota is crucial for human health, functioning as a complex adaptive system akin to a vital organ. To identify core health-relevant gut microbes, we followed the systems biology tenet that stable relationships signify core components. By analyzing metagenomic datasets from a high-fiber dietary intervention in type 2 diabetes and 26 case-control studies across 15 diseases, we identified a set of stably correlated genome pairs within co-abundance networks perturbed by dietary interventions and diseases. These genomes formed a "two competing guilds" (TCGs) model, with one guild specialized in fiber fermentation and butyrate production and the other characterized by virulence and antibiotic resistance. Our random forest models successfully distinguished cases from controls across multiple diseases and predicted immunotherapy outcomes through the use of these genomes. Our guild-based approach, which is genome specific, database independent, and interaction focused, identifies a core microbiome signature that serves as a holistic health indicator and a potential common target for health enhancement.

Presented by

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Antibiotic Effects on Gut-Derived Short Chain Fatty Acids in Healthy Human Volunteers

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Introduction: Short-chain fatty acids (SCFAs) are bioactive metabolites produced by colonic anaerobic commensals and play a central role in gut health, immune regulation, and colonization resistance. Disruption of SCFA homeostasis is implicated in infectious and chronic non-communicable diseases. Antibiotics differ in their effects on SCFA production depending on anaerobic activity and colonic exposure. This study characterized longitudinal SCFA profiles across three antibiotic classes in healthy adults.

Methods: Healthy volunteers received a 10-day course of vancomycin, omadacycline, or moxifloxacin, with stool collected at baseline, daily during therapy (days 1-10), and during follow-up (days 13 and 30). SCFAs were quantified by LC-MS/MS. Gut microbial composition was assessed by 16S rRNA gene amplicon sequencing, with quantitative PCR used to measure select taxa and genes involved in SCFA synthesis. Longitudinal SCFA trajectories were analyzed on the log10 scale using linear mixed-effects models with subject-level random intercepts to account for repeated measures.

Results: Baseline SCFA concentrations were similar across treatment arms. SCFA trajectories differed significantly by antibiotic and time, with modest between-subject variability (intraclass correlation coefficient ~ 10-20%) and dominant treatment-associated effects. Vancomycin caused the steepest and most pronounced suppression of both linear and branched SCFAs, reaching a nadir during late therapy (approximately day 7) with delayed recovery, accompanied by expansion of Enterobacteriaceae and depletion of obligate anaerobes. Omadacycline and moxifloxacin produced milder declines with earlier rebound. SCFA levels recovered by day 30 across all treatment arms.

Conclusions: Antibiotics induce class-specific disruptions of gut microbial fermentation. Longitudinal SCFA profiling provides a functional readout of anaerobic microbiome injury and recovery, adding a critical dimension to antimicrobial stewardship strategies.

Funding: Paratek Pharmaceuticals

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Thursday, July 9

[VII] Anaerobes in Reproductive Health and Skin Diseases

*Indicates presenter

Deciphering the Vaginal Microbiome with Chemistry

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The human body is colonized by trillions of microorganisms that exert a profound influence on human biology, in part by providing functional capabilities that extend beyond those of host cells. In particular, there is growing evidence linking chemical processes carried out by the human gut and vaginal microbiomes to various health outcomes ranging from cancer development to preterm birth. However, we still do not understand the vast majority of the molecular mechanisms underlying how the human microbiome influences host biological processes. Major obstacles faced in surmounting this knowledge gap include the difficulty linking functions associated with the human microbiome to specific microbial enzymes and the challenge of controlling these activities in complex microbial communities. This talk will discuss my lab's efforts to characterize vaginal microbial metabolic activities that are linked to human health, understand their relevance in human vaginal microbiomes, and to develop tools and approaches to control microbial metabolism in this environment. Gaining a molecular understanding of microbial metabolic activities in the vaginal microbiome will not only help to elucidate the mechanisms by which these organisms affect host health but should also enable efforts to improve reproductive health outcomes.

Presented by **EMILY BALSKUS**

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Sex Hormone Networks Underlying Sexual Dimorphism in C. *difficile* Infection

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Clostridioides difficile infection is the leading cause of hospital-associated antibiotic-related diarrhea. Susceptibility to infection and disease severity vary with factors such as antibiotic exposure, age, immune status, and sex. Epidemiological data consistently report a higher incidence of infection in females than males. The purpose of this study was to determine how estrous cycle stage and associated hormone networks influence *C. difficile* infection outcomes in female mice. We show that female mice infected with spores from the hypervirulent *C. difficile* strain R20291 develop more severe disease than male mice, thereby reproducing the sexual dimorphism observed in human infection.

To examine the influence of the estrous cycle, female mice were challenged with spores at defined stages. We observed a time-delayed effect: animals in proestrus at any point during disease progression developed severe symptoms one to two days later, whereas those in estrus were protected. This suggests that estrous timing relative to infection strongly modulates disease outcome.

Network analyses revealed that pre-infection levels of prolactin, IL-1β, G-CSF, KC, and IgG2b correlated with disease severity one day after infection. Similarly, progesterone, luteinizing hormone, IgG1, eotaxin, and IP-10 predicted severity with a two-day delay. Early post-infection immune effectors formed a hormone-independent network correlating with concurrent disease, while early follicle-stimulating hormone, together with IL-1β and KC, contributed to a network influencing recovery.

In conclusion, female sex hormones affect *C. difficile* infection progression by modulating immune responses both before and during infection. These findings validate the murine model for studying sexual dimorphism and may guide future hormone-based therapeutic strategies.

Presented by
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Cutibacterium acnes Disrupts *S. lugdunensis* Biofilm: Inhibition of Purine Biosynthesis Decreases Autolysis and Extracellular DNA Release

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Cutibacterium acnes is an anaerobic bacterium and a major component of the human skin microbiome. We have previously showed that molecules produced by *C. acnes* inhibit biofilm formation of *Staphylococcus lugdunensis*, an opportunistic pathogen, without affecting its planktonic growth. The purpose of this study was to investigate the mechanisms behind *C. acnes* activity against *S. lugdunensis*. We first observed that different *C. acnes* strains can produce molecules, present in their cell-free supernatants, with similar antibiofilm activity against *S. lugdunensis* in a dose-dependent manner. We then detected that these molecules affect the initial steps of *S. lugdunensis* biofilm formation, as well as its ability to adhere to A549 epithelial cells and HaCaT keratinocytes. Transcriptomics of *S. lugdunensis* was performed by RNASeq, revealing genes that were differentially expressed in the presence of *C. acnes* molecules. Interestingly, most of the *S. lugdunensis* genes involved in purine biosynthesis were significantly repressed, while genes involved in repression of autolysis (lrgA and lrgB) were highly induced. Addition of guanine, but not other purines, reverted the activity of *C. acnes* molecules on biofilm inhibition as well as induction of lrgA gene expression. Finally, we detected that *C. acnes* molecules inhibit *S. lugdunensis* autolysis and extracellular DNA release. Altogether the data suggest that *C. acnes* molecules inhibit *S. lugdunensis* biofilm formation through the depletion of the guanine intracellular pool leading to decreased autolysis. The resulting decrease of extracellular DNA release is crucial for biofilm formation. Ongoing research is currently focusing on identifying the bioactive molecules responsible for *C. acnes* activity.

Presented by
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Thursday, July 9

[VIII] Anaerobes in Gut-Brain Axis

*Indicates presenter

Enteric Glia Response to *C. difficile* Toxin-Induced Cell Death

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Clostridioides difficile continues to be an urgent threat in the USA and the major cause of nosocomial diarrhea worldwide. *C. difficile* infection (CDI) presents with diarrhea and other local or systemic symptoms depending on disease severity and host inflammatory response. Gastrointestinal (GI) complications post-CDI have been reported in humans and reproduced in a mouse model, suggesting involvement of the enteric nervous system (ENS) during infection. Enteric glia, an ENS component, plays an important role in regulating GI motility and is susceptible to *C. difficile* toxins A (TcdA) and B (TcdB). *C. difficile* toxins and CDI induce enteric glia death in vitro and in vivo, releasing proapoptotic molecules and activating proinflammatory signaling (such as adenosine/A2BR, ATP/P2X7R, S100B/TLR4, S100B/RAGE, or TRPV4). We recently found that human, mouse, and rat enteric glia express all known receptors for TcdA and TcdB, facilitating the entry of these toxins and their subsequent effects. CSPG4 is the most upregulated TcdB receptor during *C. difficile* toxin exposure and is significantly increased in colonic tissues from infected mice and humans. However, blocking CSPG4 was ineffective in reducing glial death induced by TcdB but slightly decreased cell death induced by TcdA in vitro. Real-time cell death assay revealed that increased cell death occurs at later exposure to TcdA and TcdB and is mediated by host factors, including signaling pathways involving S100B (via RAGE and TLR4), a marker of disease severity in humans, P2X7R, TLR4, and A2BR. Manipulating one or more of these pathways impacts GI function in the mouse model. Overall, our findings show that complex host-derived signaling is involved in enteric glia injury and ENS dysfunction post-*C. difficile* infection.

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Systems Ecology of the Human Microbiome in Parkinson's Disease: from Measurements to Treatments

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Parkinson's disease (PD) is associated with gut microbiome dysbiosis, but the functional and ecological consequences of these alterations, alongside their therapeutic implications, remain unclear. We apply a systems ecology framework integrating multi-omics measurements with mechanistic and interventional studies to dissect microbiome contributions to PD.

Using paired nasal-wash and stool samples and longitudinal cohorts spanning prodromal idiopathic REM sleep behaviour disorder (iRBD), de novo and treated PD, and matched controls, we identified conserved gut taxonomic shifts in iRBD and PD, with depletion of short-chain fatty acid (SCFA) producers (*Roseburia*, *Blautia*, *Faecalibacterium*) and enrichment of *Akkermansia*, *Methanobrevibacter* and *Hungatella*; mild cognitive impairment did not add a distinct microbial signature. Integrated metagenomics, metatranscriptomics, metaproteomics and meta-metabolomics revealed elevated faecal beta-glutamate, reduced secondary bile acids and SCFAs, and decreased expression of flagellar assembly, chemotaxis and bacterial microcompartments. Weighted gene co-expression network analysis highlighted depletion of functional hub genes and a global loss of taxonomic diversity of gene expression in PD. Mechanistically, in alpha-synuclein-overexpressing mice, fibre deprivation combined with the bacterial amyloid curli accelerated enteric and brain alpha-synuclein pathology and motor decline. Building on this, we have recently uncovered small amyloidogenic proteins encoded by overlooked microbial small open reading frames that cross-seed alpha-synuclein, defining a new class of microbiome-borne PD risk factors. Translating these insights, a randomised controlled trial in 74 PD patients showed that resistant starch supplementation expanded *Faecalibacterium* and SCFAs, suppressed opportunistic pathogens, modulated the systemic inflammatory proteome (APOA4, HSPA5), and reduced clinical symptoms.

Together, these findings establish a multi-scale systems ecology of the PD microbiome, ranging from amyloidogenic peptides to dietary fibre, and define rational, mechanism-anchored microbiome-targeted interventions.

Presented by
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Anaerobic Strain-Specific Differences Shape Neurologic Health and Disease

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The gut microbiome, which is comprised largely of anaerobic bacteria, can have a profound effect on neurologic health and disease by modulating immunity and by the production of neuroactive and immunomodulatory metabolites. Studies have found microbiota differences across multiple neurologic diseases, including multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease. Furthermore, studies using germ-free or antibiotic treated mice show altered disease progression. It is well established that anaerobic bacteria can have strain-specific effects in health and disease, including the production of beneficial metabolites or the presence of toxins. However, the strain-specific role of anaerobes in the gut microbiome is under characterized. In multiple sclerosis, we found that *Akkermansia* was linked to beneficial clinical outcomes in our patient population. We isolated *Akkermansia* from MS patients and found that a novel strain, BWH-H3, had a greater protective effect in the animal model of MS. This was linked to a reduction in inflammatory IL-17+ gamma-delta T cells, a decrease in microglial inflammatory genes, and a production of beneficial metabolites. In Alzheimer's disease, studies have found that *Bacteroides* correlates with clinical markers of AD, but there are conflicting results across studies which may be due to strain-specific effects. We found that the Type strain of Bacteroides fragilis increases amyloid-plaques by impairing microglial-mediated amyloid clearance. To uncover strain-specific effects, we isolated *Bacteroides* strains from healthy controls (HC) and AD subjects, then tested their ability to induce amyloid uptake. We found that a HC-derived *B. dorei* stimulated amyloid uptake in vitro where an AD-derived *B. dorei* failed to induced uptake. *In vivo*, AD-*B. dorei* increased amyloid plaques, impaired cognition, downregulated microglial phagocytosis genes while up-regulating inflammatory microglial genes. HC-B. dorei improved cognition, increased neuronal layer thickness, and increased microglial phagocytic genes. These strains differed in genes involved in LPS biosynthesis and in the production of neurotoxic / neuroprotective metabolites. Both studies in *Akkermansia* in MS and *Bacteroides* in AD highlight the power of using closely related microbial strains with differential effects to uncover potential mechanisms by which anaerobes affect neurologic diseases.

Presented by
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Thursday, July 9

[IX] *C. Diff* I: Clinical and Epidemiology Updates

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Enterococcus Colonization Impact on *C. difficile* Infection

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Enterococcus colonization with *Enterococcus faecalis* and *Enterococcus faecium* is a known risk factor for *Clostridioides difficile* infection (CDI). Enterococcus co-colonization increases *C. difficile* virulence and toxin production through cross-feeding. However, current disease severity definitions do not take into account the patients' microbiota composition or functionality. This study aimed to investigate the impact of Enterococcus colonization on disease severity.

This was a case-control study of adult patients hospitalized with CDI from two health systems (14 hospitals) in Houston, TX USA (2016-2025). Patients with severe CDI were matched to nonsevere patients on age and immunocompromised status. Stool samples were collected from hospitalized CDI patients, cultured for *C. difficile*, and stool underwent DNA extraction for qPCR analysis of *E. faecalis* and *E. faecium* and shotgun sequencing. Patients with *Enterococcus* spp. quantity > 10 were designated as highly colonized. Targeted metabolomics were completed by LC-MS/MS. Disease severity and CDI classification were defined according to the 2017 IDSA/SHEA clinical guidelines. A total of 190 patients with CDI were included (female: 54.7%, age>65: 60%, hospitalacquired CDI: 42%; CDI initial episode: 87%). In this population, 61% of patients were highly colonized. Patients with severe disease had a higher percentage of high pathogenic *Enterococcus* spp. colonization, though not statistically significant (67.5% vs 58.7%, p=0.07). Metabolomic analysis showed that patients with severe disease had higher amounts of ornithine in the stool than patients with nonsevere disease. The gut microbiota is a diverse environment with many inter-species interactions. The arginine deaminase pathway in *Enterococcus* spp. produces extracellular ornithine which can be utilized by *C. difficile*. We observed that patients with severe disease had higher concentrations of ornithine in their stool samples than patients with nonsevere disease. While a trend is observed between *Enterococcus* spp. colonization and CDI disease severity, it is highly likely that other microorganisms contribute to this association.

Presented by **TARYN EUBANK**

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Impact of a Defined Bacterial Consortium on Intestinal Homeostasis in Patients with *C. difficile* Infection

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VE303 is an investigational 8-strain bacterial consortium designed to restore a gut environment that is inhospitable to C. difficile. In the CONSORTIUM Study (NCT03788434), VE303 was associated with lower recurrence rates versus placebo and early microbiota recovery. We assessed host response by profiling gene expression of exfoliated gut cells in stool.

Patients (N=79) completing treatment for lab-confirmed CDI were randomized to placebo or VE303 once daily for 14 days. Exfoliated human RNA profiles were generated by Foli-seq in stool samples collected during dosing. Reads were aligned to the human transcriptome (GRCh38) and normalized to housekeeping genes to generate expression profiles of immune and gut physiology genes (n=2346 genes). Differential expression by CDI outcome and VE303 strain colonization status was assessed by linear mixed models; gene set enrichment analysis (GSEA) identified pathway enrichment.

At Day 14, recurrent cases showed upregulation of several genes (p-adjusted<0.05) including DUOXA2, a marker of perturbed mucosal homeostasis. GSEA showed that pathways involved in inflammatory programs and epithelial stress signaling (e.g., IFN-γ/IFN-α responses, apoptosis) were enriched in both recurrent and low-colonized groups (p-adjusted<0.1). No enrichment was observed in non-recurrent and high-colonized groups. VE303 strain colonization correlated with reduced expression of transcriptional programs associated with epithelial stress, mucosal injury, and inflammation, supporting its protective role against CDI recurrence.

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Presented by **RAJITA MENON**

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Global Epidemiology of Reduced Susceptibility to Vancomycin and Impact of Mutations on Treatment of *C. difficile* Infection

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Clostridioides difficile infection (CDI) is a leading cause of antibiotic-acquired diarrhea. Vancomycin (VAN) is widely used in the treatment of CDI, however, the mechanisms, global epidemiology, and clinical implications of VAN resistance in *C. difficile* remain poorly characterized. Our recent study has reported that the reduced susceptibility to VAN (VRS; MICs=4-16 mg/L; EUCAST breakpoint >2 mg/L) decreases both initial clinical response and sustained cure rates. This finding challenges the established view that VRS isolates are unlikely to impact therapy since oral VAN reaches colonic concentrations of 550-2200 mg/L. Herein, we describe genetic mechanisms and global epidemiology of VRS and model their impact on treatment outcomes in mice. From a cohort of 598 CDI patients (Houston), VRS occurred at 29.1%. Mutations in VanSR, two-component system that regulates the $vanG_{Cd}$ confer VAN resistance by making Lipid II with D-Ala-D-Ser motif which is 7-fold more refractory to VAN binding. Sequencing of *vanSR* identified mutations associated with VRS (VanR [$E_{11}K$, $T_{15}A$, $E_{209}G$]; VanS [$I_{118}N$, $I_{118}T$, $K_{119}E$, $T_{149}I$, $R_{314}C$ and $L_{325}F$]). These isolates exhibit constitutively expressed $vanG_{cd}$ likely augmenting Lipid II bearing D-Ala-D-Ser. The analysis of the global epidemiology of the VanSR SNPs showed 5 VanRS SNPS (VanR [$E_{11}K$, $T_{115}A$]; VanS [$I_{118}T$, $T_{149}I$, $R_{314}C$) identified in Houston are also present among 30,720 genomes in Enterobase and were predominant in North American & European isolates, suggesting restricted geographical dissemination. Interestingly, 49.4% of VRS clinical isolates did not carry resistance SNPs in vanRS. We next examined the clinical relevance of VRS bearing mutations in *vanSR* using murine severe CDI colitis model. Two VRS isolates from patients failing VAN monotherapy were compared with a related susceptible clinical isolate and R20291. All isolates were similarly pathogenic in mice. However, VAN failed to treat mice infected with VRS isolates while had better outcomes in mice infected with susceptible isolates. Pharmacokinetic revealed declining colonic VAN concentration between doses and time-kill assays showed a time-dependent bactericidal effect of VAN against *C. difficile*. Together these findings uncover a more complex pattern of resistance evolution than previously understood and confirm VRS isolates impair treatment outcomes for VAN.

Presented by **CHETNA DUREJA**

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Friday, July 10

[X] *C. Diff* II: Clinical and Epidemiology Updates

*Indicates presenter

Using Live Imaging to Investigate *C. difficile* Spore Outgrowth

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To survive adverse conditions some cells form dormant spores which can revive in response to signals encountered in the gut of a potential host. The dynamics of and mechanisms by which a spore returns to a fully active vegetative cell (outgrowth), are poorly understood but critical to CDI. Here, we investigate the role of several metabolic pathways and a set of spore-specific proteins in outgrowth by live time-lapse imaging of outgrowing *C. difficile* spores. We generated several mutant strains of *C. difficile* with deficiencies in two key areas: Stickland fermentation and small, acid soluble proteins (SASPs). The Stickland pathways, which *C. difficile* cells use to generate ATP and oxidize NADH to NAD+, are important to vegetative growth, pathogenesis, and competition with the gut microbiome. SASPs are small proteins produced by many spore-forming bacterial species that play roles in regulating sporulation, protection from UV radiation, chemicals, and, after degradation, provide a source of free amino acids for the outgrowing cell. We took, live, time-lapse phase contrast images on agarose pads in an anaerobic chamber. Differences between mutant strains outgrown on rich medium show that spores deficient in all 4 SASP genes outgrow at lower frequencies, at later times, and many of the resulting newly outgrown vegetative cells are non-viable. In contrast, spores deficient in reductive Stickland genes outgrow similarly to wild-type spores, with no detected delays or deficiencies. Remarkably, after only one round of cell division, the resulting daughter cells show growth phenotypes like those of mid-exponential vegetative control samples of each strain. These results indicate that none of the disrupted Stickland pathways play a major role in outgrowth, which could mean that ATP generation is not a limiting factor of spore revival in rich medium, or that reviving spores preferentially rely on other metabolic pathways. The outgrowth defects observed in the SASP-deficient strains, including strains that encode SASPs which can no longer bind DNA, indicate that degradation of SASPs into amino acids serves a purpose other than simply an amino acid reservoir, as hypothesized for many organisms.

Presented by
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Microbiota Driven Bile Acid Metabolism and how it Affects *C. difficile* Colonization and Virulence

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Clostridioides difficile infection (CDI) is most triggered in individuals who have recently taken antibiotics, which leads to depletion of the gut microbiota and, importantly, to depletion of the associated pool of microbially modified bile acids (BAs), including deconjugated and dehydroxylated (e.g., secondary) BAs. Restoring the gut microbiota's protective functions—particularly its ability to synthesize secondary BAs —remains a critical goal for targeted rCDI therapeutics. Recent findings from our laboratory and others have led to a radical shift in our understanding of microbial BA alterations and their interactions with *C. difficile*. Specifically, beyond the traditionally recognized microbially altered BAs, our findings revealed that Bile Salt Hydrolases (BSHs), enzymes produced by the gut microbiota, generate hundreds of unique microbially conjugated bile acids (MCBAs) through the addition of free amino acids to a BA core. The specific effects of these MCBAs on *C. difficile*, the host, and the microbiota are unexplored. To investigate the biological relevance of MCBAs, my group treated mice with a cocktail of lactobacilli BSHs to alter the BA pool and prevent CDI. We found that BSH treatment increased MCBA levels while reducing *C. difficile* spore germination and colonization in mice, however it did not prevent disease. Furthermore, we confirmed that MCBAs directly inhibited *C. difficile* spore germination, growth, and toxin expression *in vitro*. In parallel, a FMT study in patients with rCDI revealed a shift from high primary MCBA abundance pre-FMT to high secondary MCBA abundance post-FMT, suggesting an association between BSH activity, secondary MCBAs, and FMT success. The most abundant post-FMT BSHs were encoded by members of the Lachnospiraceae Family. We plan to leverage Lachnospiraceae BSHs to control secondary BA and MCBA production as a strategy to modulate *C. difficile* colonization and toxin-mediated inflammation, which has the potential to reveal new therapeutic targets for prevention and treatment of CDI and other intestinal diseases.

Presented by **CASEY THERIOT**

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C. difficile Synthesizes Immunosuppressive Indol-3-Acetic Acid Through a Novel Pathway Inducible by Select Members of the Microbiome: a Potential Mechanism of C. difficile Persistence

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The enteric anaerobe *Clostridioides difficile* (Cd) is one of healthcare's most pervasive pathobionts, causing a near 500,000 infections in the United States annually. The most challenging aspect of Cd infection (CDI) is Cd's ability to persist in the host and cause recurrent infection (rCDI), the mechanisms of which are still poorly understood. As colonization of Cd is largely dependent on the permissibility of the host microbiome, we investigated how different members of the rCDI colon microbiota contribute to Cd persistence. Using untargeted liquid-chromatography mass-spectrometry, we measured the metabolic changes of Cd when grown in different microbially-conditioned sterile supernatants. We identified that certain members of the host-microbiome induce a metabolic shift in Cd, marked by a 5-fold increase in the production of the tryptophan metabolite, indole-3-acetic acid (IAA). As indole derivatives such as IAA are known to have immunomodulatory effects, we asked how IAA may impact Cd infection. Our CDI murine data demonstrate that 50 mg/kg IAA concentrations promote decreased Cd clearance from the colon and concomitant decreases in CD8+ T cell activation. In preliminary human data, 75% (3/4) of rCDI patients from our small prospective cohort had detectable levels of IAA in their stool, compared to just 13% (2/15) of those who resolved CDI, further suggesting IAA contributes to Cd persistence (p=0.0882 at present).

To begin to understand why only certain microbes promote IAA production by Cd, we screened a Cd transposon mutant library for genes required for IAA synthesis. We propose a previously undescribed IAA synthesis pathway in which IAA is made by Cd directly from indole-pyruvate via indole-pyruvate oxidoreductases. Our data suggest that this operon is regulated by SigL, as it contains a putative upstream SigL binding site as well as an upstream SigL

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C. difficile Synthesizes Immunosuppressive Indol-3-Acetic Acid Through a Novel Pathway Inducible by Select Members of the Microbiome: a Potential Mechanism of C. difficile Persistence

(CONT.)

regulatory protein (EBP). We hypothesize the transcription of this EBP, and thus the regulation of the IAA operon, is influenced by a currently uncharacterized microbial small molecule(s).

This work provides early evidence of cross-phylum communication between Cd and select microbiota members that induce host mucosal immunosuppression, suggesting a targetable molecular mechanism for Cd persistence.

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Structural Insight into how *Clostridioides difficile* Spores Sense Bile Acid Germinants.

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Clostridioides difficile is a gastrointestinal pathogen that is the leading cause of nosocomial infections in the United States and many developed countries. C. difficile infection begins when its metabolically dormant spores encounter gut-specific small molecules that trigger the process of germination. This process occurs via a unique mechanism in C. difficile because it lacks the transmembrane receptors conserved in almost all spore-forming bacteria and requires two signals to initiate germination: a bile acid germinant and an amino acid or divalent cation co-germinant. While two soluble pseudoproteases, CspC and CspA, were initially implicated in sensing germinant and co-germinant signals, respectively, we previously showed that CspC modulates the sensitivity of C. difficile spores to both signals. Here, we show that CspC forms a stable complex with CspA and that this complex, rather than the individual proteins, is responsible for integrating germinant and co-germinant signals. By determining the crystal structure of the CspC:CspA heterodimer, we have identified interactions at the CspC:CspA interface that regulate the sensitivity of C. difficile spores to germinant signals. By co-crystallizing the CspC:CspA heterodimer with a competitive germinant inhibitor, CaPA, we also identified a bile acid binding pocket within the CspA subunit near the CspC:CspA interface. Our mutational analyses suggest that a flexible helix within the binding pocket transmits the bile acid signal to residues at the CspC:CspA interface that regulate germinant sensing. Specifically, mutations within this binding pocket decrease germinant sensitivity, whereas mutations at the heterodimer interface either expand or alter germinant specificity. Collectively, we show the first structure of a competitive germinant inhibitor bound to the CspC:CspA heterodimer and provide novel insight into how the CspC:CspA heterodimer functions as the bile acid germinant sensor in C. difficile.

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Friday, July 10

[XI] Biology of Fusobacterium

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Outer Membrane Protein FomA is Essential for Membrane Integrity and Nutrient Uptake in *Fusobacterium nucleatum*

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Fusobacterium nucleatum is a strictly anaerobic bacterium associated with periodontal disease and systemic conditions, including preterm birth and colorectal cancer. FomA, the most abundant outer membrane protein in *F. nucleatum*, has been characterized as both an adhesin and a porin. While its role in adhesion is well established, its physiological function as a porin remains poorly understood. Although previous studies suggested that *fomA* is nonessential, here we demonstrate that FomA is required for cell viability. Using a conditional depletion system, we show that loss of FomA results in severe growth defects, cell elongation, and disruption of outer membrane integrity. Mechanistically, FomA functions as a major nutrient uptake channel mediating the transport of glutamate, lysine, and fructose—key carbon and energy sources for F. nucleatum. Depletion of FomA leads to nutrient limitation and significant physiological stress. Interestingly, acetate supplementation partially restores growth in FomA-depleted cells. We further demonstrate that acetate catabolism is repressed in the presence of preferred nutrients such as glutamate, lysine, or peptides, and that acetate uptake occurs independently of FomA, revealing metabolic flexibility under nutrient-limited conditions. Together, these findings redefine FomA as an essential metabolic gatekeeper required for nutrient acquisition and outer membrane homeostasis, providing new insight into the physiology and pathogenic potential of this important anaerobe.

Presented by
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Identification of a Fusobacterial RNA-Binding Protein Involved in Host Small RNA-Mediated Growth Inhibition

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Host-derived small RNAs are emerging as critical regulators in the dynamic interactions between host tissues and the microbiome, with implications for microbial pathogenesis and host defense. Among these, transfer RNA-derived small RNAs (tsRNAs) have garnered attention for their roles in modulating microbial behavior. However, the bacterial factors mediating tsRNA interaction and functionality remain poorly understood. In this study, using RNA affinity pull-down assay in combination with mass spectrometry, we identified a putative membrane-bound protein, annotated as P-type ATPase transporter (PtaT) in *Fusobacterium nucleatum* (*Fn*), which binds *Fn*-targeting tsRNAs in a sequence-specific manner. Through targeted mutagenesis and phenotypic characterization, we showed that in both *Fn* type strain and a clinical tumor isolate, deletion of ptaT led to reduced tsRNA intake and enhanced resistance to tsRNA-induced growth inhibition. Global RNA sequencing and label-free Raman spectroscopy revealed the phenotypic differences between *Fn* wild type and *PtaT*-deficient mutant, highlighting the functional significance of PtaT in purine and pyrimidine metabolism. Furthermore, AlphaFold 3 prediction provides evidence supporting the specific binding between PtaT and Fn-targeting tsRNA. By uncovering the first RNA-binding protein in *Fn* implicated in growth modulation through interactions with host-derived small RNAs (sRNAs), our study offers new insights into sRNA-mediated host-pathogen interplay within the context of microbiome-host interactions.

Presented by
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Gene Regulatory Networks and Stress Adaptation in *Fusobacterium nucleatum*

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Fusobacterium nucleatum is a common member of the human oral microbiome, but it is also increasingly recognized as an opportunistic pathogen capable of disseminating to distant body sites. In particular, *F. nucleatum* can colonize tumor tissues, where it has been linked to cancer progression, immune modulation, and poor clinical outcome. Despite this biomedical relevance, we still know surprisingly little about how this phylogenetically distinct bacterium regulates gene expression and adapts to the diverse environments it encounters within the host.

To address this gap, we generated a molecular framework for understanding gene regulation in *F. nucleatum*. We mapped fundamental features of fusobacterial gene expression, including transcriptional organization and a diverse set of previously uncharacterized small noncoding RNAs (sRNAs). Because genetic manipulation has historically limited mechanistic studies in fusobacteria, we also developed new genetic tools that enable targeted functional analysis in *F. nucleatum*.

Using these tools, we identified a stress-response pathway controlled by the alternative sigma factor σ^E, a transcriptional regulator commonly associated with bacterial envelope and cell-surface stress responses. We further uncovered two σ^E-associated small RNAs that contribute to this response, highlighting an additional layer of post-transcriptional regulation. Comparative analysis revealed both conserved principles and unexpected differences between the fusobacterial σ^E pathway and the better-characterized σ^E systems of model bacteria.

Presented by
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Gene Regulatory Networks and Stress Adaptation in *Fusobacterium nucleatum*

(CONT.)

Together, our work establishes new experimental and conceptual resources for studying gene regulation in *F. nucleatum*. More broadly, it shows how an understudied member of the Fusobacteriota uses regulatory strategies to respond to stress and adapt to host-associated environments. These findings provide a foundation for future studies into fusobacterial physiology, host colonization, and potential vulnerabilities that may be exploited to limit disease-associated *F. nucleatum*.

Presented by
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Friday, July 10

[XII] Anaerobes in Animals and the Environment

*Indicates presenter

Of Man and Other Animals: the Same Clostridial Diseases?

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Several clostridial diseases affect humans and other animals, while a few of them affect only humans, and others affect only other animals. Amongst those that affect both humans and other animals are those produced by *Clostridium perfringens* type A and C, *Clostridium novyi* type A, *Paraclostridium sordellii*, *Clostridioides difficile*, *Clostridium septicum*, *Clostridium tetani* and *Clostridium botulinum*. Examples of diseases that affect several animal species but not humans are *Clostridium chauvoei*, *Clostridium colinum*, *Clostridium piliforme* and *Thomasclavelia spiroformi*. Of particular relevance is *C. difficile* associated disease (CDAD), which affects, in addition to humans, many other animal species, including horses. In the latter, *C. difficile* is one of the most important casues of enterocolitis, with diarrhea and high mortality. Although as in humans, equine CDAD is frequently linked to hospitalization and antimicrobial treatment, cases without these risk factors also occur. A retrospective review of 59 equine CDAD cases (2014-2024) submitted for post-mortem examination was performed. The cohort included 34 adults, 16 juveniles, and 9 neonatal foals. Inclusion criteria were detection of TcdA and/or TcdB and/or positive culture for *C. difficile*. Complete necropsies were performed, HE and Gram-stained tissue sections were examined, toxin (A/B) detection was performed by ELISA, and selective culture for *C. difficile* was performed. The most common clinical presentation was acute diarrhea and/or colic (71%). Twelve per cent of cases had received antimicrobial treatment and 14 % had a history of hospitalization. The main gross and histopathological findings were necrohemorrhagic colitis (25%), enterocolitis (19%), enteritis (19%) and typhlocolitis (17%). Four cases (7%) were positive for *C. difficile* by culture alone, 14 (24%) by toxin detection alone, and 41 (70%) by both methods. Clinical findings and gastrointestinal lesions were similar across groups: colic and gastrointestinal lesions were observed in 30% of culture-positive/toxin-negative cases, in 32% of toxin-positive/culture-negative cases, and in 39% of cases positive by both methods, indicating similar clinical and pathological profiles regardless of the diagnostic assay used. Equine CDAD shows heterogeneous clinical and pathological presentations and limited agreement between culture and toxin detection. These findings support the complementary use of both diagnostic approaches for accurate diagnosis and improved epidemiological interpretation.

Presented by *Contact*

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From Microbes to Medicine: Decoding the Pathobiome Across Translational Disease

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Canine obesity is associated with reduced health span and alterations in the gut microbial pathobiome, yet current interventions such as dietary caloric restriction and exercise often fail to yield sustained metabolic improvements. This study evaluated whether fecal microbiota transplantation (FMT) from lean fecal donors modulates the pathobiome and improves metabolic outcomes in obese dogs undergoing dietary weight management. In a blinded, placebo-controlled crossover clinical trial, 23 obese dogs were randomly assigned to dietary management alone or combined with oral FMT or placebo capsules over 24 weeks. Shotgun metagenomic sequencing was performed longitudinally, with taxonomic and functional profiling using MetaPhlAn4 and HUMAnN. All obese dogs, regardless of treatment, experienced significant weight loss over time (Two-way ANOVA, $p<0.0001$), with no apparent treatment impact. However, obese dogs receiving FMT from lean donors demonstrated a significant improvement in insulin sensitivity, evidenced by a positive repeated measures correlation between fasting insulin-to-glucose ratio and weight loss ($p.adj<0.05$). Microbial community structure shifted significantly following FMT (PERMANOVA & Pairwise Adonis2; $p< 0.05$), while minimal changes were observed with diet alone. A literature-guided functional analysis identified 2,000 obesity-associated KEGG Orthologs (KOs), including 130 core baseline KOs. Of these using MaAsLin2, 15 KOs related to carbohydrate metabolism, amino acid transport, and energy pathways were significantly depleted only in FMT-treated dogs by week 24. Notably, two phosphotransferase system KOs (KO2759, KO2760) showed FMT treatment-specific associations with insulin sensitivity. These findings indicate that targeted modulation of the canine obesity associated gut pathobiome via lean donor FMT improves metabolic function in obese dogs despite limited shifts in core microbial functions, highlighting its therapeutic potential.

Presented by *Contact*

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Synergistic Interactions Among *Fusobacterium necrophorum, Bacteroides pyogenes, and Porphyromonas levii* in Bovine Metris

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Bovine metritis is associated with uterine microbiota dysbiosis characterized by the proliferation of gram-negative anaerobes, including *Fusobacterium necrophorum*, *Bacteroides pyogenes*, *and Porphyromonas levii*. However, the interactions among these opportunistic pathogens and their roles in disease development remain unclear. This study aimed to characterize their interactions and contributions to metritis.

F. necrophorum, *B. pyogenes*, and *P. levii* isolated from the uteri of cows with metritis were cultured anaerobically in chopped meat carbohydrate broth. Bacterial growth, coaggregation, biofilm formation, endotoxin production, protease activity, adhesion to bovine endometrial epithelial (BEND) cells, and cytotoxicity were evaluated under mono-, dual-, and triple-species conditions.

Among the three species, *F. necrophorum* grew the fastest and acted as a key species that coaggregated with *B. pyogenes* and *P. levii*, promoting multi-species biofilm formation and bacterial adhesion to BEND cells. Growth of *B. pyogenes* was enhanced by metabolites from *P. levii*, whereas metabolites from *F. necrophorum* delayed *P. levii* growth. The triple-species biofilm produced the greatest biomass, with *P. levii* in the basal layer and *F. necrophorum* and *B. pyogenes* co-localizing in the upper layer, reflecting the strong matrix-producing ability of *P. levii*. Endotoxin levels were highest in *F. necrophorum* and *P. levii*, and protease activity was highest in *P. levii* under mono-species conditions, with no synergistic effects in co-culture. Notably, co-culture with *B. pyogenes* reduced endotoxin levels and attenuated the cytotoxicity of *P. levii*. These findings suggest that these pathogens interact to enhance uterine persistence rather than disease severity, likely contributing to dysbiosis and chronic inflammation.

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Friday, July 10

[XIII] Anaerobic Resistance and Novel Treatments

*Indicates presenter

Antimicrobial Resistance Mechanisms Driving the Discovery of New Therapies Against *C. difficile*

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Clostridioides difficile infection (CDI) remains a leading cause of antibiotic-associated diarrhea and healthcare-associated infection, with nearly 500,000 infections annually in the United States. CDI treatment is at an important crossroads as strains with reduced susceptibility to metronidazole, vancomycin, and fidaxomicin are increasingly recognized. However, the true scale and clinical impact of this problem remain underdefined, in part because anaerobic susceptibility testing is not routinely incorporated into CDI diagnostic workflows. This seminar will examine mechanisms of resistance to first-line CDI therapies and their implications for clinical management and therapeutic discovery. Metronidazole resistance has been linked to genetic changes that increase 5-nitroimidazole reductase activity, whereas vancomycin resistance can arise through multiple mechanisms, including mutations in the VanSR two-component regulatory system that induce the *vanGCd* operon and alter peptidoglycan precursor structure from D-Ala-D-Ala to D-Ala-D-Ser. These mechanisms have been detected across diverse genetic backgrounds and geographic settings. Fidaxomicin resistance is primarily associated with mutations in RNA polymerase, underscoring target-based adaptation rather than MarR-mediated regulation. Together, these examples highlight the evolutionary plasticity of *C. difficile* under antimicrobial pressure and the need for improved resistance detection, clinical interpretation, and alternative treatment strategies. This talk will conclude by discussing our ongoing discovery and development platform for narrow-spectrum antimicrobials and non-antibiotic approaches for CDI management.

Presented by
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Antimicrobial Resistance and the Gut Microbiome

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Antimicrobial resistance (AMR) is recognized by the WHO as one of the three major threats to human health. In recent decades, AMR organisms have globally increased in prevalence, and pathogens such as the facultative anaerobic Enterobacterales have become almost completely resistant to available antibiotics. There is a dire need for novel mitigation strategies that prevent the emergence of antimicrobial resistance, reduce its impact, and improve its clinical management. Colonization and persistence with multi-drug resistant organisms (MDRO) such as carbapenem-resistant Enterobacterales (CRE) happens in the context of the gut microbiome, which contains a complex metagenome that may harbor a reservoir of antimicrobial resistance genes, and may promote or suppress the growth of MDRO. In this talk we will first review the recent evolution of carbapenem resistance genes and the new challenges it poses for the successful clinical management of CRE. We will then discuss how CRE become established in the gut microbiome, in particular in immune-compromised patients such as solid-organ transplant recipients. Lastly, we will review longitudinal dynamics of intestinal MDRO colonization as well as the protective role of anaerobes such as Bacteroides and Blautia spp. in MDRO colonization resistance. These observations may pave the way for future non-antibiotic interventions to reduce the burden of AMR in vulnerable patients.

Presented by
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Nutritional Suppression of *C. difficile*

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Clostridioides difficile, a major cause of antibiotic-associated diarrhea, is suppressed by the gut microbiome through multiple mechanisms; however, exploitative competition by gut microbes may be a driving mechanism. Through meta-analysis of 12 human studies aided by machine learning approaches, we designed a synthetic fecal microbiota transplant (sFMT1) by reconstructing microbial networks negatively associated with *C. difficile* colonization in diverse human populations. This lab-built 37-strain consortia formed a functional community suppressing *C. difficile* in vitro and in animal models. Using sFMT1 as a tractable model system, we interrogated its function via a multi-omics approach and iterative reduction of community complexity. We demonstrated that both bile salt deconjugation and 7α-dehydroxylation were not determinants of sFMT1 function. A single strain of *Peptostreptococcus russellii (anaerobius)* which performs reductive Stickland fermentation of proline, a pathway of competitive nutrient utilization, was both necessary and sufficient to suppress *C. difficile* infection, replicating the efficacy of a healthy human fecal transplant in a gnotobiotic mouse model. In complementary studies in humans and humanized gnotobiotic mice, we discovered that short-term, but not long-term, caloric restriction and/or highly host-bioavailable diets impart increased resistance to *C. difficile* infection. In addition to reduced intake of digestion-resistant protein, we identified and isolated a limited set of diet-enriched microbes which could suppress *C. difficile* infection in the absence of caloric restriction by further limiting amino acid availability. Increased understanding of how caloric restriction modulates infection resistance is allowing the design of precision diet interventions that are nutritionally sufficient for the host but insufficient for *C. difficile* to effectively decouple host and microbial energetic states. Taken together, these observations support a path through which *C. difficile* may be targeted by combined microbial and dietary interventions seeking to exploit its dependency on reductive Stickland fermentation of proline in the gut.

Presented by **JORDAN E. BISANZ**

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Poster Abstracts

Poster Abstracts are divided by day, with Young Investigator Competition abstracts listed first on Wednesday, July 8. Within each group, they are alphabetized by last name of the poster presenter.

Wednesday, July 8

Young Investigator Poster Abstracts

*Indicates presenter

Wednesday, July 8

Young Investigator Poster Abstracts *(CONT.)*

*Indicates presenter

Wednesday, July 8

Young Investigator Poster Abstracts *(CONT.)*

*Indicates presenter

Alzheimer's Disease-Derived *Phocaeicola dorei* Contributes to Cognitive Decline in a Strain-Specific Manner

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The human gut microbiota undergoes substantial compositional and functional shifts with aging and Alzheimer's disease (AD). While associations between bacterial species and disease risk are increasingly recognized, functional heterogeneity at the strain level remains poorly understood. Here, we examined whether strain-level variation within *Phocaeicola dorei*, a common human gut commensal bacterium that is elevated in AD, differentially influences host immunity and neurodegenerative outcomes.

We compared a *P. dorei* strain isolated from an individual with AD to a strain isolated from a healthy control. In vitro, the healthy control strain enhanced macrophage-mediated amyloid beta (Aβ) uptake, whereas the AD-derived strain failed to stimulate phagocytosis. In vivo colonization of APP/PS1 mice revealed that the AD-derived strain impaired spatial memory in females, increased Aβ plaque burden, and reduced microglial density in affected brain regions, accompanied by elevated peripheral inflammatory T cell responses. Microglial transcriptomic profiling indicated that colonization with the AD-derived strain altered metabolic activation states.

Whole-genome sequencing identified the absence of wbyH, encoding a putative O-antigen biosynthesis protein, in the AD-derived strain. Consistent with this genomic difference, SDS-PAGE analysis revealed structural divergence in lipopolysaccharide (LPS) between strains, supporting prior evidence that LPS structure influences AD-related pathology. Metabolomic profiling further showed that the healthy control strain retained the capacity to synthesize vitamins B1 and B6, whereas the AD-derived strain lacked these pathways and was associated with depletion of neuroprotective lipids and accumulation of neurotoxic metabolites in the brain.

Together, these findings demonstrate that strain-specific genomic and metabolic features of gut bacteria critically shape host immune responses and disease-relevant outcomes, underscoring the importance of strain-level resolution in microbiome research and highlighting the gut microbiota as a modifiable contributor to Alzheimer's disease progression.

Presented by **CARTER ABBOT**

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Fusobacterium nuclueatum-Derived Short-chain Fatty Acids Alter Expression of Tumor-Associated Genes in Colorectal Cancer Cells

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Cystic fibrosis (CF) is a systemic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an anion channel that regulates movement of chloride and bicarbonate ions. CFTR is recognized as a tumor suppressor gene, as its dysfunction alters cellular proliferation pathways, contributing to tumorigenesis. Low tumoral CFTR expression has been associated with increased colorectal cancer (CRC) severity and mortality. CF patients exhibit decreased gut microbial diversity and an enrichment of CRC-associated bacteria, including *Fusobacterium nucleatum* (Fn). However, Fn's interactions with the CF gut epithelium, and whether it synergizes with CFTR dysfunction, is poorly understood.

To test the hypothesis that Fn contributes to tumorigenesis via *CFTR* repression, we examined how *Fn*-derived metabolites, including short-chain fatty acids (SCFAs), affect colonic epithelial cells. Treatment of Caco-2 cells with *Fn* cell-free supernatant led to a significant downregulation of *CFTR* mRNA expression. Notably, CFTR repression was attributable to the lowest molecular weight fraction of the supernatant and was unaffected by heat treatment, suggesting regulation by small, heat-stable metabolites such as SCFAs. To test this, cells were treated with increasing concentrations of butyrate, propionate, or a combination of both SCFAs. Treatment with 10 mM and 15 mM butyrate resulted in significant repression of *CFTR* expression. RNA-sequencing revealed that SCFAs broadly modulate the Caco-2 transcriptome, including upregulation of several tumor-associated genes and transcription factors (e.g. *MYC*, *CEACAM5*). SCFAs downregulated the expression of multiple ion channels (e.g. *TRPM6*, *ABCC9*), as well as genes involved in cellular metabolism and non-coding RNAs.

Overall, this work suggests that *Fn*-derived SCFAs can influence tumorigenic pathways, providing new insight into host-microbe interactions and linking CFTR regulation, microbial metabolism, and CRC risk.

Presented by
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Synergistic Commensal Interactions Confer Asymptomatic Protection Against *Clostridioides difficile* Infection In Vivo

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Clostridioides difficile infection (CDI) is a major cause of nosocomial diarrhea in the U.S., affecting ~500,000 people annually and causing ~30,000 deaths. Current standard treatments with antibiotics often fail to prevent recurrence, and fecal microbiota transplants (FMT) pose potential unknown risks. Members of the Phylum *Firmicutes* have been shown to provide resistance to CDI, but the key species and mechanisms remain unclear. Here, we colonized C57BL/6 germ-free (GF) mice with two healthy donor-derived *Firmicutes* strains, *Paraclostridium* strain 691 and *Hungatella* strain 659. GF mice mono-colonized with P. 691 survived C. difficile challenge but displayed mild diarrheal symptoms and weight loss, whereas H. 659-colonized mice became moribund within 36 hours of *C. difficile* challenge. Interestingly, co-colonization of GF mice with both strains led to asymptomatic protection, accompanied by a significant reduction in *C. difficile* burden at 36 hours post-infection. Depletion of *C. difficile*-essential nutrients like Stickland amino acids and the enrichment of short chain fatty acids (SCFAs) have been associated with resistance to CDI, but the causative inter-microbial interactions remain understudied. Metabolic analyses of mouse cecal contents revealed cross-feeding of Stickland amino acids between the strains and an increased production of SCFAs from amino acid utilization. *H.* 659 significantly enriched Stickland amino acids in mono-colonized mice. These excess amino acids were depleted by *P.* 691 in the co-colonized mice correlating to significantly enriched SCFA levels. These findings suggest synergy between the two strains in enhancing resistance against CDI and demonstrate essential commensal interactions in a simplified mouse model, offering insights into the use of targeted microbiota-based therapies for CDI.

Presented by
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Disarm *Fusobacterium* in Colorectal Cancer: Inhibition of Amyloid-Like FadA Reduces Tumor Burden and Enhances Efficacy of Immunotherapy

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Fusobacterium nucleatum (Fn), a gram-negative oral anaerobe, is an opportunistic pathogen associated with a wide spectrum of human disease, including periodontal disease, preterm birth, and various Gi disorders and cancers. *Fn* presence in colorectal cancer (CRC) correlates with adverse clinical outcomes such as enhanced tumor aggressiveness, advanced disease stages, reduced sensitivity to chemotherapeutic agents and reduced overall survival. The amyloid-like form of the *Fn* adhesin FadA, expressed under disease and stress conditions, plays a key role in stimulating CRC growth. The prevalence of amyloid-like FadA expression is the highest among the strains isolated from CRC (71.4%), followed by those from the intrauterine cavity (33.3%), inflammatory bowel disease (IBD, 27.3%), and oral cavity (7.1%). We screened a panel of ring-fused 2-pyridone compounds to identify candidates that could inhibit FadA function without bactericidal effects or host toxicity.

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(CONT.)

This screen revealed PS598, a tricyclic compound that selectively disrupted amyloid-like FadA and abrogated its function. In cell attachment assays, PS598 inhibited the binding to, and growth stimulation of, HCT116 cells by *Fn* expressing amyloid-like FadA, but not the non-FadA-expressing isolates. In the murine CT26 CDX tumor model, *Fn* significantly stimulated tumor growth. PS598 administration dampened this aggressive growth. Intriguingly, the combination of PS598 with anti-PD1 antibody further inhibited tumor growth. These findings support the potential of targeting *Fn*-host interactions as a novel adjunctive strategy to improve the efficacy of immunotherapy in CRC implicated with *Fn*.

Visualizing the Inerplay Between Toxin and Sporulation Gene Expression During Murine Infection in *Clostridioides difficile*

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Clostridioides difficile is the most common cause of healthcare-associated infections in the United States. The *C. difficile* spore form is responsible for transmitting disease, while its glucosylating toxin-producing cells are primarily responsible for causing disease. Both sporulation and toxin genes are heterogeneously expressed within a given population *in vitro*, creating a subset of cells expressing sporulation genes and another expressing toxin genes. Analyses of these two subpopulations at the single-cell level during growth on certainlaboratory media revealed that there is little overlap between sporulation and toxin gene expression, suggesting that *C. difficile* establishes a "division of labor" between sporulation and toxin production. While this "division of labor" could benefit *C. difficile* at the population-level because toxin gene expression and sporulation are tightly regulated and metabolically intensive processes, it is unclear whether this "division of labor" occurs during host infection. Building on previous lab work showing that toxin genes are heterogeneously expressed during murine infection using chromosomally encoded fluorescent reporters, we have developed a novel triple reporter system to visualize the overlap between sporulation and toxin gene expression at the single-cell level. We are using this system to determine if a "division of labor" in transmission and virulence gene expression occurs during a murine infection and what factors affect the distribution of their gene expression at the single-cell level. For example, we have introduced the reporter system into toxin-null and inducible toxin strains. This work will provide novel insight into determining the interplay between toxin and sporulation gene expression and the dynamics of their expression at the single-cell level during infection.

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Prevalence of *Clostridioides difficile* and its Association with Intestinal Dysbiosis in Healthy Cats and those with Diarrhea

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Clostridioides difficile can colonize the intestines of cats, but its role as a pathogen in feline disease remains unclear. Although *C. difficile* prevalence is associated with intestinal dysbiosis in humans and dogs, this has not yet been described in cats. We aimed to assess the prevalence of *C. difficile* in cats and its association with intestinal dysbiosis. For this, 336 cat feces from four cohorts were evaluated retrospectively: 83 healthy cats (HC); 94 cats with chronic enteropathy without a history of antibiotic use in the four months prior to enrollment (CE; food-responsive, n=20, and steroid-responsive, n=74); 59 cats with exocrine pancreatic insufficiency (EPI); and 100 samples from cats with unknown clinical status submitted to a diagnostic laboratory. Samples were evaluated by qPCR for *C. difficile* and for intestinal dysbiosis using the Dysbiosis Index (DI). *C. difficile* was detected in 0% (0/83) of HC, 7.5% (7/94) of cats with characterized CE, 14% (8/59) of cats with EPI, and 13% (13/100) of cats with unknown clinical status. Cats positive for *C. difficile* had significantly increased DI (median[min-max]: 2.4 [-0.3 - 4.4] vs. -0.3 [-4.4 - 4.1]) compared to cats testing negative (Mann-Whitney, P<0.001). Odds ratio of *C. difficile* detection was 8 times higher in cats with increased DI (DI>0) compared to normal DI (DI<0) (OR 95% CI: 2.4 - 24.9, Fisher's exact, P<0.001). Cats positive for *C. difficile* had a significant reduction in the bile acid-converting *Peptacetobacter hiranonis* (median [min-max]: 0.3 (0.1 - 6.6) vs. 5.5 (0.1 - 7.4)) compared to cats testing negative (Mann-Whitney, P<0.001). Odds ratio of *C. difficile* detection was 10 times higher in cats with reduced *P. hiranonis* compared to those within reference interval (OR 95% CI: 3.8 - 23.7, Fisher's exact, P<0.001). Colonization by *C. difficile* was associated with intestinal dysbiosis and reduced bile acid-converting *P. hiranonis* in cats. The overall prevalence of *C. difficile* was 8.3% (28/336); however, in cats with CE, prevalence did not appear to be influenced by treatment modality.

Presented by
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Sneathia Vaginalis is Resistant to Killing by Neutrophils and complement

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Purpose: *Sneathia vaginalis* is a Gram-negative anaerobe that is strongly associated with adverse pregnancy outcomes, including chorioamnionitis, preterm premature rupture of membranes, and preterm birth. Rarely, it can cause bacteremia and sepsis, suggesting that it may be equipped with defenses against bloodborne immune components. *S. vaginalis* is a diderm, and diderms are often killed directly by complement through formation of the microbial attack complex (MAC) in the outer membrane unless they express virulence factors that prevent complement deposition and/or MAC formation. In addition to direct complement killing, complement and specific antibodies can opsonize bacteria, promoting phagocytosis and killing by neutrophils. The goal of this study was to determine the sensitivity of S. vaginalis to complement and neutrophil-mediated killing.

Methods and Results: Complement killing was measured by incubating ~10^6 bacteria/ml with 30 mg/ml complement from human serum (25 CH50 units/mL) in fetal bovine serum (FBS) for 1 hr. Bacteria were enumerated by the drip plate method. A urinary tract infection isolate of *Escherichia coli* exhibited 0.009% survival (standard deviation = 0.003%), while *S. vaginalis* strain SN35 exhibited 32% survival (standard deviation = 4%) survival. To assess killing by opsonophagocytosis, we incubated *S. vaginalis* with either pre-immune rabbit serum, or antiserum to CptA, a pore-forming toxin expressed on the outer membrane of *S. vaginalis*. We have previously reported that this antiserum binds effectively to the surface of *S. vaginalis*. The antiserum-exposed bacteria were incubated at a 1:2 ratio with primary human neutrophils in RPMI containing 10% non-heat-inactivated FBS for 1 hr. There was no significant killing of *S. vaginalis* by neutrophils, even when the bacteria had been opsonized by anti-CptA antiserum.

Conclusion: These findings suggest that *S. vaginalis* is resistant to direct complement-mediated killing and to neutrophil-mediated killing, even when opsonized. This property likely contributes to its ability to cause bacteremia and disseminated infections. Understanding how *S. vaginalis* evades immune defenses could help to inform future therapeutic strategies.

Presented by **GILBERT GLAGO**
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Persistence of *Clostridioides difficile* Spores in the Intestinal Mucosa During Prolonged Vancomycin Treatments

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Our prior work correlated *Clostridioides difficile* spore adherence and internalization into intestinal epithelial cells with disease recurrence. In this work, we developed an R-CDI murine model mimicking human R-CDI to explore how *C. difficile* spores persist in the intestinal mucosa.

The SHEA standard of care recommends 10 days of vancomycin treatment; however, prior murine models of R-CDI used a 5-day vancomycin regimen, resulting in 100% recurrence rates. Hence, to improve the translational potential of our R-CDI murine model and gain clinically relevant insight into *C. difficile* persistence, we extended vancomycin treatment to 15 days. Results demonstrated that *C. difficile*-infected mice treated with 5-days of vancomycin led to 100 % recolonization, whereas extending vancomycin treatment to 15 days led to ~25% of the mice being re-colonized with the infective strain. Stool vancomycin remained at inhibitory concentrations throughout the course of vancomycin treatment, and both 5- and 15-day vancomycin regimens led to similar gut microbiota composition and beta diversity, suggesting that the *C. difficile* spore reservoir was being depleted. Using confocal microscopy, we monitored adhered and internalized spores at the peak of CDI, and after 5 and 15 days of vancomycin treatment. We observed that adhered and internalized spores to cecal and colonic tissues at the peak of CDI significantly decreased after 5 days, yet remained at detectable levels after 15 days of vancomycin. Strikingly, aggregations of *C. difficile* spores resembling microcolony-like structures were evident at the peak of CDI and persisted throughout both vancomycin treatments, albeit at lower densities.

Together, these results demonstrate that extending vancomycin treatment significantly reduced the severity and incidence of R-CDI in our murine model, likely due to partial depletion of the persisting spore reservoir during treatment, suggesting unknown spore-renewal mechanisms.

Presented by **TREY HEJTMANCIK**
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The Development of Anti-Germination Compounds as a Prophylactic for *Clostridioides difficile* Infections

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Clostridioides difficile, the causative agent of *C. difficile* infections (CDI), is the leading cause of nosocomial-acquired antibiotic-associated diarrhea, pseudomembranous colitis, and toxic megacolon. The US alone approximates an average of 500,000 annual CDI cases with an associated cost of $5 billion.

To survive in the external environment, *C. difficile* produces metabolically inactive dormant spores that are resistant to environmental insults. *C. difficile* spores serve as the infectious and transmissible unit for CDI. *C. difficile* spores are primarily transmitted via the fecal-oral route.

Following ingestion, *C. difficile* spores are transported through the gastrointestinal (GI) tract where they are exposed to bile salts that signal spores to germinate into toxin-producing cells. Following germination, metabolically active vegetative *C. difficile* cells come to reside in the colon where they reproduce and produce toxins responsible for the onset of CDI symptoms.

Because *C. difficile* spore germination is required for the establishment of disease, obstruction of this process inside an infected host has the potential of being used for CDI management. Synthetic bile salt compounds with amide bonds have previously shown to be potent inhibitors of *C. difficile* spore germination in vitro and in vivo. These anti-germination compounds were able to prophylactically protect mice and hamsters from CDI. However, due to a susceptible amide bond, similar to one in naturally occurring bile salts, the first generation of anti-germination compounds were inactivated by GI bacterial bile salt hydrolases.

To address stability issues, a new generation of nonhydrolyzable bile salt analogs (NHBS) has been designed. These NHBSs lack the labile amide bond and thus are expected to be resistant to in vivo deconjugation. In this proposed study, the efficacy of the next generation of anti-germination compounds has been tested. Our data shows these NHBSs are potent inhibitors of *C. difficile* spore germination in vitro, with inhibitory activity in the low micromolar range. Additionally, ex vivo studies have demonstrated these NHBSs have multi-day resistance to GI bacterial modifications.

Presented by **EFREN HEREDIA**

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Reproducibility and Variability Assessment of the Crystal Violet Biofilm Assay in *Clostridioides difficile*

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Crystal violet staining is the most widely used quantitative assay for bacterial biofilm analysis; however, reproducibility across microtiter plate formats, surface chemistries, and biological replicates remains a persistent challenge, particularly for anaerobic pathogens such as *Clostridioides difficile*, whose biofilm formation varies substantially across strains and growth conditions. Here, we present a comprehensive reproducibility assessment of the crystal violet biofilm assay in *C. difficile*, systematically evaluating the effects of plate format, surface treatment, and isolate-level heterogeneity on assay variability. Biofilms of reference strains (CD630 and R20291) and fifteen clinical isolates were grown under anaerobic conditions in 24-, 48-, and 96-well microplates and quantified spectrophotometrically across three independent experiments. Reproducibility was assessed using within-experiment and across-experiment coefficients of variation (CV%). Tissue-culture-treated 96-well plates consistently reduced variability relative to untreated plates, while increasing well size markedly improved reproducibility, with 24-well tissue-culture-treated plates yielding the lowest CV% values. Linear mixed-effects modeling confirmed these trends and revealed substantial experiment-to-experiment noise in small-volume plate formats that limited statistical power. Variance partitioning across the fifteen-isolate panel demonstrated that isolate-level biological heterogeneity accounted for approximately 20% of total within-experiment CV variance, whereas experiment-level variance was negligible, indicating strong day-to-day assay stability under optimized conditions. Integration of within- and across-experiment variability into a composite reproducibility score further distinguished highly stable isolates (CV% < 10%) from intrinsically variable biofilm formers (CV% > 30%). Collectively, these findings demonstrate that crystal violet biofilm assay reproducibility in *C. difficile* is governed by both technical parameters and isolate-specific biofilm physiology, and provide an evidence-based framework for assay standardization and improved cross-study comparability.

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Investigating the Role of Gut Microbiome-Produced Sulfated Phenols in Autism Spectrum Disorder

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Background: Autism spectrum disorder (ASD) affects approximately 1 in 31 children in the United States and is associated with lifelong disability in social communication and behavior. Emerging evidence suggests that the gut microbiome may contribute to ASD pathogenesis through production of neuroactive metabolites. In particular, the gut-derived metabolite, 4-ethylphenyl sulfate (4EPS) is elevated in ASD patient serum, induces ASD-like behaviors in mice, and promotes aberrant neuronal demyelination. While the final biosynthetic step of 4EPS production – sulfonation of its precursor 4-ethylphenol (4EP) – has been assumed to be exclusively catalyzed by host sulfotransferase (SULT) enzymes, this has not been rigorously verified.

Purpose: We show that human gut bacteria can catalyze phenolic sulfonation to contribute to levels of 4EPS in the host and characterize the arylsulfate sulfotransferase (ASST) enzyme responsible for activity.

Methods and Results: Using targeted metabolomics, we identified that an ASD-associated *Clostridium* species can convert 4EP and other phenols to their corresponding phenolic sulfates in culture. This species encodes a candidate ASST that we hypothesized was responsible for its activity. Heterologous expression of the *asst* gene yielded a purified protein with sulfonation activity, while Clostron-mediated gene disruption in the native strain abolished sulfonation ability. In vitro cell culture studies using colonic epithelial cells as well as in vivo studies in mice suggest that 4EPS sulfonated by ASST-containing bacteria may elevate levels of this compound in the gut lumen as well as in systemic circulation.

Conclusions: Our findings challenge the view that 4EPS sulfonation is exclusively host-driven and support a model in which microbiome-encoded ASST activity contributes to 4EPS levels in host tissues. Ongoing work is focused on identification of additional bacteria with ASST activity, metagenomic analyses of ASD datasets for *asst* prevalence, and behavioral studies in mice following colonization of the *asst* wild-type and knockout *Clostridium* strains. Such studies will lay the groundwork for future microbiome-targeted interventions for ASD.

Presented by
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Epigenetic control of Cell Fate: DNA Methylation Regulates Spoiie Expression to Promote Sporulation and Developmental Flexibility in *Clostridioides difficile*

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Methylation of the bacterial genome is a widespread phenomenon and can regulate gene expression, though the mechanisms underlying epigenetic regulation are often poorly understood. In *Clostridioides difficile*, the orphan methyltransferase CamA promotes sporulation, a process critical for persistence and transmission of this nosocomial pathogen. While CamA was previously shown to enhance activation of the sporulation-specific sigma factor, σ^F, its direct regulatory targets were unknown. Here, we show that methylation of a single CamA motif upstream of the sporulation gene *spoIIE* is sufficient to promote its transcription, increasing the frequency of σ^F activation and enhancing sporulation efficiency. Surprisingly, the CamA-dependent increase in spoIIE expression also drives premature activation of σ^F in predivisional cells in over a third of the sporulating population. While σ^F activation in the forespore irreversibly commits a cell to completing sporulation, cells that activate σ^F in the predivisional cell can abort the sporulation program and resume vegetative growth. Thus, CamA enhances sporulation without compromising a population's capacity to adapt to fluctuating environmental conditions. Finally, we find that CamA provides a significant fitness advantage during infection that is largely independent of its role in sporulation, suggesting it regulates additional genes that confer important functions during infection. These findings highlight CamA, which is highly specific to *C. difficile*, as a multifaceted regulator of bacterial fitness during infection and, therefore, a promising antimicrobial target.

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Forazemin: Precision Antibiotic Targeting of Periodontal Pathobionts

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Periodontal disease affects over 1 billion people worldwide and is driven by dysbiosis of anaerobic oral biofilms dominated by pathobionts such as *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Treponema denticola*; however, selective antibiotics for these pathogens are lacking. Current therapies rely on broad-spectrum antibiotics, including metronidazole and clindamycin, which indiscriminately disrupt the oral microbiome rather than selectively eliminate key pathobiont anaerobes. In contrast, selective antibiotics spare pioneer biofilm-forming commensals such as *Streptococcus spp.* (*S. oralis* and *S. sanguinis*) and *Lactobacillus spp.*, which produce antimicrobial metabolites that competitively exclude anaerobic pathogens, maintain eubiosis, and reduce recurrence of disease. Here, we present forazemin as a promising therapeutic against periodontal anaerobic pathobionts. Antimicrobial activity was assessed in vitro against representative oral anaerobes and commensal bacteria, followed by efficacy evaluation in a rat ligature-induced periodontitis model. In vitro, forazemin selectively eliminated Gram-negative anaerobic pathobionts, including *Fusobacterium*, *Porphyromonas*, *Aggregatibacter*, *Treponema*, and *Prevotella spp.*, with minimal inhibitory concentration (MIC) values ranging from 0.015 to 1 µg/ml, while sparing beneficial streptococci and lactobacilli. In a rat ligature-induced periodontitis model, oral administration of forazemin cleared periodontal pathogen burden, promoted oral tissue regeneration and decreased inflammation, while reducing systemic immunoinflammatory indices, including the systemic immune-inflammation index (SII) and systemic inflammatory response index (SIRI). Histopathological analyses revealed that forazemin treatment reduced inflammatory infiltrates, promoted reorganization of connective tissue, and improved alveolar bone repair. These findings support forazemin as a precision antibiotic that targets periodontal pathogens while preserving the beneficial oral microbiome.

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The Immunogenic Response of a Chimeric Protein Vaccine Targeting *Clostridioides difficile*

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Clostridioides difficile is a gram-positive, spore-forming, toxin-producing anaerobe and a leading cause of antibiotic-associated diarrhea. *C. difficile* infections (CDI) can range from mild diarrhea to pseudomembranous colitis, and if left untreated, can lead to death. The standard treatment options for acute CDI are limited to three antibiotics: Metronidazole, Vancomycin, or Fidaxomicin. All these antibiotics have limited effectiveness due to the continual disruption of the normal gut flora, leading to high rates of recurrent infections. To combat this problem, research is exploring vaccine-based prevention, with prior findings showing that immunization with a portion of the *C. difficile* toxin TcdB can provide protective immunity in mice. However, toxin-based vaccination approaches have had limited success due to persistently high bacterial burdens after challenge. This study aims to evaluate the immunogenicity of a chimeric protein vaccine that combines a TcdB-derived toxin component with a *C. difficile* surface-associated protein, with the goal of eliciting both anti-toxin and antibacterial immune responses. To achieve this aim, mice were assigned to 5 different treatment groups and vaccinated every 7 days for a total of 3 vaccinations. Blood serum samples were collected before each vaccination and 1 week post last vaccination. Serum samples were analyzed for antigen-specific IgG responses. Additional analysis of cecum samples was performed to assess secretory IgA responses. Lastly, splenocytes were isolated to examine the expression levels of 5 target genes corresponding to T cell activation. Together, these analyses are designed to characterize the systemic and mucosal immune responses elicited by this chimeric vaccine candidate and to inform its potential utility in *C. difficile* vaccine development.

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Akkermansia muciniphila Reduces Amyloid-Beta Pathobiology by Modulating Peripheral Immunity in a Mouse Model of Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by amyloid-beta (Aβ) plaque deposition and neurofibrillary tangles. Alterations in the gut microbiome have been reported in AD patients, and microbiota-based interventions can influence disease progression. *Akkermansia muciniphila*, comprising 1–4% of the healthy gut microbiota, is reduced in AD and proposed as a next-generation probiotic. Recent reports have demonstrated its immunomodulatory and barrier-protective effects in neurodegenerative diseases. In this study, we evaluated the therapeutic potential of *A. muciniphila* BWH-H3 in APP/PS1 mice. For this purpose, seven-week-old mice were gavaged orally with *A. muciniphila* for six weeks, stratified by sex and parental inheritance of the transgene. At 3.5 months, female APP/PS1 mice developed more severe Aβ pathology than males, consistent with controls. Notably, *A. muciniphila* significantly reduced hippocampal Aβ plaque burden only in female APP/PS1 mice with paternal transgene inheritance; other groups showed no significant effect. Immunological analysis revealed reduced IFN-γ production by $CD8^+$ T cells in gut-draining lymph nodes of treated females. Microglial bulk transcriptomics showed modulation of immune pathways, including upregulation of neutrophil degranulation, NOD-like receptor (NLR) signaling, and GM-CSF signaling. These findings suggest that *A. muciniphila* exerts sex- and inheritance-dependent effects on AD pathology through immune activation and microglial reprogramming, promoting early Aβ plaque reduction.

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United We Stand: Uncovering The Interactions Between *Cutibacterium acnes* and *Staphylococcus lugdunensis* in Dual-Species Biofilms

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Our skin is home to trillions of microbes that together form the skin microbiome. Prosthetic joint infections (PJIs) pose a costly threat to both individuals and the healthcare system. These infections are persistent and require revision procedures, which increase the surgical failure and mortality rates of prosthetic replacements. One main virulence factor in these infection-causing bacteria is their ability to form biofilms, which protect them from antibiotic treatments and the host immune response. Understanding the mechanisms that govern biofilm formation is critical in treating these infections. *Staphylococcus lugdunensis* and *Cutibacterium acnes* are common skin commensals that are frequently co-isolated from chronic infections of prosthetic devices. We theorized that these bacteria synergistically interact with each other to enhance the severity of polymicrobial infections. We found that *C. acnes* and *S. lugdunensis* interact with each other to increase biofilm formation compared to their respective monocultures. This phenotype is found to be specific to this combination of species. We found that both species need to be metabolically active and in physical contact to produce the phenotype. We also found that this increase in biofilm is not due to an increase in number of cells, but by an overproduction of matrix by *S. lugdunensis* in response to *C. acnes* exposure. Interestingly, unlike *S. lugdunensis* biofilms, polysaccharide is the main component of the mixed biofilm matrix. Gene expression analysis revealed that co-culture with *C. acnes* induces extracellular polysaccharide gene expression in *S. lugdunensis*. Our data indicate a novel mechanism by which these species are able to persist in medical devices and cause infections.

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Murine Model of Antibiotic-Associated *Staphylococcus aureus* Gastrointestinal infections and Colonization

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Staphylococcus aureus is an opportunistic pathogen that can both colonize the gastrointestinal tract and cause antibiotic associated diarrhea. To develop a robust murine model for *S. aureus* gastrointestinal infection (SAGII) and colonization, mice were (a) treated with varying antibiotic regimes prior to infection, (b) infected with either a methicillin-sensitive *S. aureus* (MSSA) or a methicillin-resistant *S. aureus* (MRSA) strain, (c) challenged with different bacterial inocula (d) tested for sexual dimorphism of SAGII virulence, and (e) tested for macronutrient effects on SAGII onset and virulence. Antibiotic-treated male mice (but not female mice) were highly susceptible to both an MSSA and an MRSA strains. Interestingly, male mice challenged with the laboratory MSSA strain showed more severe and more prolonged SAGII symptomatology than animals challenged with the clinical MRSA strain. Diet composition significantly influenced disease outcome: a high-carbohydrate diet and a high-fat diet led to asymptomatic intestinal colonization followed by delayed SAGII sign onset in male mice. In contrast, a high-protein diet led to an early onset of SAGII signs followed by severe SAGII signs two weeks post-challenge. Furthermore, only the high-protein diet sensitized female mice to SAGII, but their symptomatology remained less severe than in male mice. We developed a robust murine model for antibiotic-associated S. aureus gastrointestinal infection and colonization. This model shows both sexual dimorphism and macronutrient preference for SAGII signs severity. Diet manipulation can also be used to establish S. aureus colonization of the GI tract. Furthermore, the SAGII murine model demonstrates essential features of S. aureus pathogenesis which could provide understanding about human gastrointestinal colonization and infection mechanisms.

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Effect of Medium Viscosity on the Growth of *Lacticaseibacillus rhamnosus*

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Lactocaseibacillus rhamnosus is a probiotic that grows within the gut mucus, a viscoelastic barrier whose physical properties influence microbial survival. While biochemical interactions with mucus are well studied, the role of mucus rheology in shaping probiotic growth and morphology remains unclear. This question becomes especially important in diseases such as inflammatory bowel disease and gastrointestinal infections, where mucus viscosity, structure, and thickness are altered. These changes can disrupt nutrient transport, increase mechanical constraints, and reorganize microbial spatial structure, potentially impairing the ability of probiotics to proliferate, colonize, and compete with pathogens.

This study examines how viscosity influences the growth dynamics and cellular organization of *L. rhamnosus*. Cultures were grown at 37 °C in media with controlled viscosities (72–259 mPa·s) at two inoculum densities to model low- and high-density colonization. Optical density measurements quantified growth, and lag time, maximum growth rate, and final cell density were determined from growth curve analysis. Cellular morphology and chaining behavior, a feature critical for gut colonization, were evaluated using phase-contrast microscopy. Increased viscosity reduced the maximum cell density in *L. rhamnosus* cultures and strongly suppressed cellular chaining, with chain frequency and size reduced by ~2-fold, while individual cell length remained unchanged. Lower-density cultures exhibited a 1.5-fold longer lag phase but achieved a 16% higher maximum growth rate and 12% greater final cell density than higher-density cultures, highlighting how viscosity and initial population size shape growth and morphology.

These findings indicate that gut viscosity plays a key role in modulating probiotic bacteria colonization. Understanding how mechanical aspects of the gut environment influence probiotic behavior can provide insight into microbial persistence in vivo. Ongoing studies are focused on real-time monitoring of *L. rhamnosus* growth in microfluidic gut-mimicking environments, and examining the role of viscoelasticity.

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Diet Composition Modulates Murine *Clostridioides difficil* Infection Onset and Severit

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Opportunistic pathogen Clostridioides difficile is the leading cause of hospital-associated antibiotic-related diarrhea. Recent evidence suggests that different macronutrient compositions of diets may elicit protective or adverse effects during C. difficile infection in rodent models. Since CDI is triggered by gut microbiome dysbiosis and diet alters gut microbial diversity, we hypothesize that dietary macronutrient concentrations influence infection onset and severity. To test this hypothesis, we fed groups of mice eleven isocaloric diets varying in protein, fat, and carbohydrate ratios. Each group was then infected with hypervirulent C. difficile spores.

Overall, dietary carbohydrates did not influence disease severity, dietary fat showed an inverse correlation with disease severity with no effect on symptom onset, and dietary protein showed a direct correlation with CDI severity and delayed symptom onset. To determine the relation between diet, gut microbiota changes, and CDI severity, we performed 16S rRNA sequencing from feces of animals fed high protein, high carbohydrate, and high fat diets. Shannon alpha diversity and Bray-Curtis NMDS beta diversity analyses show that murine gut microbiome diversity differentiates after onset of experimental diets, and microbial diversity declines after antibiotic treatment and C. difficile spore challenge across all diet groups. Gut microbiome diversity markedly increased thirty days after C. difficile spore challenge in mice treated with the standard lab diet, high carbohydrate diet, high fat diet, but not the high protein diet.

Most notably, taxonomic bar-plot and SIMPER heatmap analyses show that murine gut microbiomes treated with the high fat diet and the high carbohydrate diet resulted in greater relative abundance of bacterial phylum Verrucomicrobiota and families Lachnospiraceae and Akkermansiaceae. In contrast, the high protein diet group showed significantly less relative abundance of these gut-health protective bacteria. This study will help close the current knowledge gap regarding how diet composition affects C. difficile infection severity, prevention, and control.

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Realized Sugar Utilization by *Anaerostipes* does not Correlate to Phylogenetic Strain Variation

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Among the most prevalent bacteria within the human gut are the Lachnospiraceae, a family of anaerobes with multiple physiologically relevant functions, such as the production of beneficial short-chain fatty acids (SCFAs) that contribute to the overall health of the intestinal epithelium. Here, we investigate sugar utilization within the genus *Anaerostipes*, a member of the Lachnospiraceae known to produce butyrate, an important SCFA. Using a collection of isolates (n=18) from healthy human fecal samples (n=5), we sought to investigate strain-level diversity in the species *Anaerostipes hadrus*. Based on genomic comparisons of 85 *A. hadrus* strains, we hypothesized that more distantly related strains would exhibit more differential sugar utilization. Phylogenetic comparison of our strains (n=17) and publicly available genomes (n=68), including a type strain (ATCC29173), identified sub-clades within the species. Of note, strains from the same fecal sample (i.e., host) within our isolate collection did not always cluster into the same sub-clade, suggesting the co-existence of phylogenetically distinct strains in the same host. To relate genomic changes to actualized phenotypic growth, we grew 11 *A. hadrus* strains in basal media with free amino acids (BMAA, pH=7) in the Biolog PreBioM1 platform, which includes 30 single carbon sources. One of our isolates, CM03-47, exhibited a wider sugar utilization profile, demonstrating growth for 26/30 carbon sources tested, compared to a mean of 12 for other strains. Multidimensional clustering based on the Bray-Curtis distance metric calculated from overall sugar profiles per strain demonstrated that strains did not cluster by phylogenetic sub-clade, suggesting that phylogenetic relatedness did not concur with phenotypic sugar use. Additionally, strains that did cluster similarly still exhibited differential growth profiles, suggesting a diversity of growth patterns, even by closely related strains. Collectively, we concluded that *A. hadrus* exhibits variable intra-species sugar utilization profiles, possibly to avoid resource competition while occupying similar niches within the gut microbiome.

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Human Gut Sporobiota as Functional Drivers of Colonization Resistance to *Clostridioides difficile* Infection

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Clostridioides difficile is the leading cause of antibiotic-associated nosocomial infections. While fecal microbiota transplantation (FMT) is highly effective in treating recurrent *C. difficile* infection (rCDI), the specific microbiome-derived mechanisms underlying its success remain incompletely defined. Emerging evidence implicates gut sporobiota—ethanol-resistant *Firmicutes*—in mediating colonization resistance. Here, we evaluated sporobiota from eight healthy human donors for their ability to confer resistance to *C. difficile* in germ-free C57BL/6 mice. Mice were colonized with donor-derived sporobiota and challenged three weeks later with *C. difficile* VPI 10463 spores. Resistance was assessed by *C. difficile* burden and toxin production. Sporobiota from three donors (37.5%) conferred complete resistance, with no detectable *C. difficile* or toxin in the cecum, whereas five donors (62.5%) failed to confer resistance, resulting in asymptomatic carriage. Comparative microbiome and metabolomic analyses revealed distinct taxonomic and bile acid signatures between resistant and carrier phenotypes. Notably, resistance was associated with higher levels of deconjugated bile acids prior to *C. difficile* challenge, suggesting a pre-existing metabolic landscape that disfavors pathogen establishment. Together, these findings suggest interindividual variability in sporobiota-mediated colonization resistance and support a critical role for bile acid metabolism, particularly bile acid deconjugation, in shaping *C. difficile* outcomes. Elucidating the mechanistic links between specific microbial communities and bile acid transformations may enable the development of more targeted microbiota-based therapies to enhance FMT efficacy and prevent *C. difficile* recurrence.

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Gut Microbial Bile Acid Metabolites Influence Thymic T Cell composition and Signaling

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The gut microbiome shapes the host metabolome through microbial metabolism of dietary and host-derived compounds, producing metabolites that influence host immunity both locally and across peripheral tissues. Our laboratories have previously shown that secondary bile acids (BAs), which are bacterial metabolites of host-derived primary BAs, can modulate intestinal immunity by influencing T cell differentiation. We now report that bacterial BA metabolism can also affect extraintestinal immune tissue function, focusing on the thymus, the central organ for T cell selection and maturation. In mice, we found that secondary BAs comprise a substantial fraction of the thymic BA pool, including ^{13}C-labeled secondary BAs following oral gavage of their labeled precursors. Germ-free mice also showed rapid thymic uptake of secondary BAs and demonstrated altered thymocyte composition and selection markers after 1 week of treatment with secondary BAs. Using complementary loss-of-function and gain-of-function approaches to manipulate secondary BA levels, we further found that bacterially modified BAs regulate thymic expression of the chemokine *Ccl21*, likely in medullary thymic epithelial cells (mTECs). Given that Ccl21 is involved in progenitor recruitment, thymocyte migration to the medulla for negative selection, and egress of mature T cells, this signaling pathway may have important implications for T cell selection and adaptive immunity more broadly. Together, these findings provide initial evidence for a novel gut-thymus axis mediated by secondary BAs, through which gut bacteria may influence T cell development at its earliest stages.

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Wednesday, July 8

Wednesday Abstracts - Continued

*Indicates presenter

Wednesday, July 8

Wednesday Abstracts - Continued

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Real-World Outcomes of Fecal Microbiota Spores, Live-BRPK for Recurrent *Clostridioides difficile* Infection in Patients with Inflammatory Bowel Disease

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Recurrent *Clostridioides difficile* infection (rCDI) remains common and is associated with substantial morbidity, particularly in patients with inflammatory bowel disease (IBD) who face higher recurrence risk and complex management. Fecal microbiota spores, live-brpk (VOS) is approved for prevention of recurrent infection following standard-of-care antibiotics; however, patients with active inflammatory bowel disease were excluded from pivotal trials, limiting real-world data.

We conducted a retrospective case series of adults with IBD treated with VOS after resolution of active Clostridioides difficile infection (CDI) till February 2026. The primary outcome was recurrent infection within 8 weeks. Secondary outcomes included recurrence within 6 months, IBD flare within 12 weeks, therapy escalation, and adverse events.

Ten patients were included (median age 50 years, range 20–69; 70% female). Crohn's disease was present in 80% and ulcerative colitis in 20%. At treatment, 50% were receiving biologics and 30% corticosteroids. The median number of prior infections was 3, including one patient with at least seven episodes. All completed therapy. Recurrent infection occurred in 30% (3/10), all following subsequent systemic antibiotic exposure. No additional recurrences occurred within 6 months in patients initially achieving clinical cure within 8 weeks. Inflammatory bowel disease flare within 12 weeks occurred in 20%, and therapy escalation in 40% (one pre-planned). Mild gastrointestinal adverse events occurred in 30%. No serious infections or treatment-attributable adverse events were observed.

In this real-world IBD cohort, VOS appeared safe and was associated with sustained remission in patients without subsequent antibiotic exposure. Larger prospective studies are warranted.

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VE303 Strains Disrupt *Clostridioides difficile* Growth and Induce Transition Metal Starvation Response

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VE303 is an investigational live biotherapeutic product (LBP) designed for prevention of recurrent *C. difficile* infection (rCDI). In the phase 2 CONSORTIUM Study (NCT03788434), VE303 reduced the odds of rCDI by >80% compared with placebo. To better understand how VE303 reduces the risk of recurrence, we characterized in vitro interactions between the 8 VE303 strains and *C. difficile* in trans-well co-culture devices, direct co-culture, and spent supernatant experiments. We measured the effect of each VE303 strain on C. difficile growth, sporulation, germination, toxin production, and global gene expression. In trans-well co-culture experiments, multiple strains of VE303 reduced growth of vegetative *C. difficile* cells to a similar extent as *C. difficile* self-competition, suggesting nutritional overlap ($p < 0.05$). Many of the same strains similarly impaired growth of *C. difficile* spores. Notably, VE303-05 and VE303-07 exhibited significantly stronger inhibition than *C. difficile* self-competition ($p < 0.05$). Co-culture with VE303-06 significantly reduced TcdA production ($p < 0.05$). In spent supernatant experiments, VE303-01 and VE303-04 supernatant significantly increased *C. difficile* spore formation during stationary phase suggesting depletion of preferred nutrients ($p < 0.05$). To determine potential mechanisms of interaction, we performed direct co-culture and RNA-seq of VE303 strains and *C. difficile* at 6 hours (early stationary phase). VE303-04, VE303-05 and VE303-07 induced potent upregulation of several specific transition metal and siderophore transporters by 5- to 64-fold compared to the *C. difficile* monoculture control. VE303-05 and VE303-07 also downregulated ribosome-associated genes and ATP synthase genes. Taken together, these data suggest that VE303 operates through multiple mechanisms where different strains compete for nutrients, increase sporulation, decrease toxin production, and compete for essential transition metals that collectively may make *C. difficile* less competitive in the gut environment and ultimately prevent recurrent infection.

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The Impact of Chondroitin Sulfate on *Fusobacterium nucleatum* Growth and Virulence

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Colorectal cancer (CRC) is among the most frequently diagnosed cancers in the US, with a concerning increase in diagnoses among young people. Annually, 1.8 million new cases with over 800,000 deaths worldwide are attributed to colorectal cancer. Microbiome analyses have identified the putative oncobacterium *Fusobacterium nucleatum* in abundance in polymicrobial biofilms on the surface of CRC tumors. *F. nucleatum* in the gut promotes tumorigenesis in mouse models of CRC, although the underlining mechanisms driving tumorigenesis are unknown. Chondroitin sulfate (CS) proteoglycans are increased in abundance on the surface of CRC tumors, which are available as a nutrient source to gut microbes. We investigated the impact of CS on *F. nucleatum* in a chemically defined media and determined that chondroitin sulfate increased its growth and significantly altered its transcriptome. CS led to differentially regulated genes associated with carbohydrate import and metabolism, amino acid uptake and metabolism, transcriptional regulation, and virulence. In particular, *fap2* expression was significantly diminished in the presence of CS, while several genes of the ModR regulon were also differentially expressed. Genes linked to hydrogen sulfide production, a known carcinogen, were also induced when *F. nucleatum* was grown with CS. Although the mechanism(s) by which CS exerts these effects on the *F. nucleatum* transcriptome, we speculate that *F. nucleatum* may use monosaccharides from CS as a carbon source or recognizes CS as a host-associated signal. In future work, we will use CRISPR to construct XYLT2 deletions in cancer cell lines to abolish CS attachment to chondroitin sulfate proteoglycans. These cells will be infected with *F. nucleatum* to determine if the change in the cell glycome impacts its ability to attach, invade, and stimulate inflammation. Likewise, we will explore the microbial ecology of *F. nucleatum* with glycan-degrading members of the microbiota (e.g., *Bacteroides* spp.) with whom it co-localizes on polymicrobial tumor communities. The long-term goal is to define the nutritional determinants allowing tumor colonization to identify antagonistic interactions that can be leveraged for colonization prevention.

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Targeted Probiotic Interventions for Recurrent *Clostridioides difficile* Infection

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Clostridioides difficile infection (CDI) is a leading cause of hospital-associated disease characterized by high recurrence due to disruption of the anaerobic gut microbiota following antibiotic treatment. Current therapies reduce vegetative bacterial growth but fail to restore microbial function, increasing susceptibility to recurrent CDI (rCDI). We aimed to identify defined anaerobe-based interventions that suppress C. difficile and restore colonization resistance. In prior work, a human-origin probiotic consortium (Pro11) significantly reduced disease severity, C. difficile burden, toxin production, and intestinal inflammation in a murine CDI model, while restoring microbiome diversity and beneficial metabolites, including butyrate. To identify simplified therapeutic candidates, genome-scale metabolic modeling using MICOM was applied to simulate microbial interactions under rCDI-associated conditions. Modeling predicted that the obligatory anaerobe *Blautia wexlerae* exhibited the strongest inhibition of *C. difficile* growth, followed by a defined multi-strain combination consisting of *Bifidobacterium breve*, *Bifidobacterium longum*, and *Lactobacillus rhamnosus* (blr). These predictions were supported by anaerobic in vitro co-culture assays and validated in vivo, where both *B. wexlerae* and blr significantly reduced recurrence. These findings demonstrate that defined anaerobic bacteria can restore colonization resistance and provide protection against CDI recurrence. Targeted single-strain and multi-strain interventions represent promising strategies for developing standardized, microbiome-based therapies for rCDI.

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Taxonomic Study of Variabaculum Species: Novel Anaerobic Actinomycete Isolated from a Clinical Specimen in Japan

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Background: *Varibaculum* is a Gram-positive, non-spore-forming, non-motile bacterial genus belonging to the family *Actinomycetaceae*. To date, only a few Varibaculum species have been described, all of which were isolated from clinical specimens. Specific identification of *Varibaculum* remains challenging because conventional biochemical test systems and commercial MALDI-TOF MS databases often lack sufficient discriminatory power. This study aimed to phylogenetically analyze a clinically isolated *Varibaculum* strain, GTC20041, that could not be assigned to any known species.

Materials and Methods: Strain GTC20041 was isolated from a clinical specimen. Biochemical characterization was performed using a Rapid ID 32A system. Identification was attempted using MALDI-TOF MS. Full-length 16S rRNA gene sequence was analyzed. Whole-genome sequencing was performed, and average nucleotide identity (ANI) was used for genomic comparison with closely related species.

Results and Discussion: MALDI-TOF MS analysis yielded scores below 2.0 for all reference species, indicating unreliable species-level identification. Phylogenetic analysis showed that GTC20041 was most closely related to *Varibaculum cambriense*, with 98.6% sequence similarity. However, genome-based identification using the GTDB-tk was unsuccessful. ANI analysis revealed that GTC20041 shared an ANI value of 88.1% with *V. cambriense*. These findings indicate that GTC20041 represents a distinct taxonomic lineage within the genus Varibaculum.

Conclusion: Strain GTC20041 represents a previously uncharacterized lineage within the genus *Varibaculum*, underscoring the necessity for further taxonomic studies of clinically relevant anaerobic bacteria.

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Characterization of *Clostridium scindens* Restriction Modification Systems for the Development of a Genetic System

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Clostridium scindens is a Gram-positive, spore forming, anaerobe capable of converting primary bile acids to secondary bile acids through removal of the 7α-hydroxyl group. Accumulation of secondary bile acids has previously been reported to inhibit growth of *Clostridioides difficile* vegetative cells, protecting against C. difficile infection (CDI). Despite interest in *C. scindens*, the lack of available genetic systems makes it difficult to investigate its role in host protection against CDI or its physiology. The work in this study aimed to characterize DNA methylation patterns and restriction-modification systems in *C. scindens* to facilitate the development of a genetic system. To accomplish this, we used Pacific Bioscience's Single Molecule Real Time (SMRT) sequencing to analyze the natural methylation patterns of three *C. scindens* strains: VPI12708, C82, and L97. From these data, we identified DNA motifs that are highly methylated across all strains, several of which were palindromic and recognized by known restriction enzymes. To test our hypothesis that C. scindens is methylating its genome at these motifs, we performed a series of restriction digests using enzymes that recognized motifs identified by our methylation analysis. Testing DNA extracted from *C. scindens*, *E. coli*, and *C. difficile*, only *C. scindens* DNA was protected from degradation. Following this, two annotated methyltransferases encoded by *C. scindens* were cloned into a pET22b vector to determine if they protected at any of the identified motifs. Following induction, DNA was subjected to digestion with PvuII, which recognizes a 6 bp motif methylated in all three *C. scindens* strains. As hypothesized, one of the methyltransferases conferred protection against degradation, supporting the presence of a restriction-modification system. Through our analysis, we have begun to characterize two putative restriction-modification systems in *C. scindens*. This work may enable the development of genetic systems for *C. scindens* by imitating native methylation patterns in donor DNA, opening the door for genetic manipulation and new studies of *C. scindens* physiology and its role in preventing *C. difficile* colonization.

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Application of Click Display Technology for Rapid Protein Binder Discovery

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Click display is a novel technology that enables the development of high affinity protein binders against a specific target protein or antigen with great affinity. It overcomes the challenges of traditional *in vitro* methods, such as ribosome display and mRNA display, by generating stable, covalently bound protein-cDNA complexes within 2 hours in a one-pot reaction system. The resulting protein-cDNA complexes make click display advantageous for biopanning for antibody-like proteins.

We hypothesize that click display technology can be successfully adapted to develop custom antibody-like proteins that will be useful for various assays and protein localization within cells.

First, a synthetic gene library encoding protein scaffold variants with randomized binding regions will be designed and synthesized as double-stranded DNA templates containing the T7 promoter and ribosome binding site sequences. The reaction will be performed *in vitro* using a translation system supplemented with the modified oligo linker from a click chemistry reaction between a puromycin oligonucleotide and a cDNA synthesis primer. Following a 2-hour incubation to complete transcription, translation, and reverse transcription, the resulting protein-cDNA complexes will be subjected to repeated rounds of biopanning against a target protein. This technology allows for the rapid and stable design of custom monoclonal antibodies. These DARPin proteins will then be translationally fused to fluorescent proteins, such as mCherry, which can be used in assays such as western blotting, IP or co-IP -type experiments, etc. As an initial approach we are trying to design tags for western blotting, such as His-tag and FLAG.

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Western-Diet-Induced Gut-Micribiome-Derived Metabolites Accelerated Neuroinflammation in APP/PS1 Mice

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The gut microbiota is altered in Alzheimer's disease (AD) patients and may contribute to AD by secreting microbial metabolic product that affect immunologic and/or neuronal function. Western diet is linked to increased AD risk and has differential effects on the gut microbiota. Whether the Western diet affects AD pathogenesis via modulating microbiota metabolic function has not been extensively investigated in mechanistic animal models. We hypothesize that the Western high-fat high-sugar diet worsens AD by shifting gut microbiota metabolites. In the male APP/PS1 mice, we first identified increased amyloid plaque burden in the hippocampal region and increased CD4+IFNγ+ and CD4+T-bet+ splenic cell populations in Western-diet-fed mice (vs control-diet-fed AD male mice). Further, we identified IL1B signaling as a central upregulated node in microglia gene expression and Ingenuity Pathway Analysis, with predicted upstream contributions from upregulated ITGAV, AGT, INSR and downregulated NFAT5. Collectively, these findings indicated that Western diet has a sex-dependent effect and exacerbate amyloid pathology and promotes systemic Th1 immune activation and microglial IL-1b-centered innate inflammatory priming in APP/PS1 male mice. Next, we identified strict anaerobes *Muribaculaceae* and *Rikenellaceae* RC9 gut group increased by Western diet vs Control diet. Interestingly, we correlated the microbiota with plaque burden in the brain and identified butyrate-produces including *Lachnospiraceae*, *Butyricicoccus*, *Ruminococcaceae*, *Oscillospiraceae*, and *Rosburia* were negatively related with plaque levels in the western diet group.

Our findings implicate Western diet-driven bacterial metabolites in exacerbated inflammation in APP/PS1 male mice, and we aim to link diet-induced gut microbiota to host metabolic and inflammatory responses using multi-compartment metabolomic analyses.

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Butyrate Synergizes with Glucose to Promote Anaerobic Growth and Biofilm Formation of *Staphylococcus aureus* via Anaplerotic Metabolism

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Short-chain fatty acids (SCFAs) are abundant microbial fermentation products in anaerobic, polymicrobial environments such as the gut, where they shape community structure and constrain opportunistic pathogens, including *Staphylococcus aureus*. Typically studied in the context of airway and skin infections (e.g., abscesses, diabetic foot ulcers), *S. aureus* is an antibiotic-associated diarrheal pathogen. Nasal colonization correlates with gut residence, which can seed infections at extraintestinal sites. While SCFAs inhibit S. aureus growth, the extent to which local nutrient availability modulates this inhibition remains unclear. Here, we investigated how glucose and galactose, two sugars found in higher abundance in the diabetic gut, alter SCFA-mediated growth and biofilm phenotypes in *S. aureus*. Wild-type JE2 and isogenic regulatory and biofilm-associated mutants were grown in LB supplemented with sodium butyrate or sodium propionate, with or without glucose or galactose. Growth kinetics, biofilm formation, and hierarchical clustering analyses were used to assess mutant- and media-dependent responses. Both SCFAs significantly inhibited anaerobic growth and biofilm formation; however, sugar supplementation partially alleviated this inhibition. Glucose more effectively restored growth and biofilm formation than galactose, particularly during butyrate exposure. In contrast, galactose-supported growth recovery was limited and revealed distinct clustering patterns among regulatory mutants. Together, these findings demonstrate that carbon source availability critically shapes *S. aureus* tolerance to microbiota-derived SCFAs. This metabolic context may influence *S. aureus* fitness and persistence within anaerobic, polymicrobial niches, with implications for understanding how gut-associated metabolites regulate pathogen behavior in complex microbial communities. Further, a clearer understanding of how *S. aureus* colonizes the SCFA-rich gut will allow for the development of rationally designed consortia for its competitive exclusion, diminishing the risk of severe infections.

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Preservation of Gut Microbiota and Low Recurrence Rates in Patients Treated with CRS3123 Compared to Vancomycin in a Phase 2 Clinical Trial for *C. difficile* Infection

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The purpose of this study was to determine the effect of CRS3123, a novel narrow-spectrum antibiotic that inhibits type 1 methionyl-tRNA synthetase, on the gut microbiota of patients in a phase 2 trial for the treatment of *C. difficile* infection (CDI).

Fecal microbiomes were profiled by 16S rRNA sequencing and metagenomics and analyzed for alpha diversity (Faith's Phylogenetic Diversity) and beta diversity (Weighted Unifrac distances) between CDI and healthy state. Fecal metabolites were measured by LCMS and GCMS.

Alpha diversity of the gut microbiome was significantly less impacted between day 1 and test of cure (TOC, day 12-15) in patients treated with CRS3123 compared to vancomycin. Beta diversity showed significantly less dysbiosis on day 4 of treatment and at TOC with CRS3123 compared to vancomycin. *C. difficile* decreased in all patients by day 4 of treatment. The relative abundance of beneficial gut microbes such as *Bifidobacterium* and *Bacteroides* increased in the CRS3123 groups but decreased in the vancomycin group. *Enterococcus spp*, including VRE, were eliminated during treatment with CRS3123, consistent with the potent activity of CRS3123 against enterococci, while vancomycin increased VRE. CRS3123 had a positive impact on the gut microbiome recovery, such as enrichment of glycan degradation (fiber breakdown) and beneficial secondary bile acid biosynthesis (e.g. lithocholic acid) pathways in CRS3123 compared to vancomycin. The observed CDI recurrence rates at day 40 were 4% (1/26) in the combined CRS3123 groups and 23% (3/13) in the vancomycin group.

In conclusion, CRS3123 better preserved and restored the gut microbiota in CDI patients, and resulted in higher rates of sustained clinical cure, which is likely attributable to the narrow spectrum of CRS3123. The multi-omics data, combined with the good safety and efficacy results, warrant further testing of CRS3123 in phase 3 clinical trials.

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Lineage-Dependent Distribution of the Vang-Like Operon and Implications for Vancomycin Resistance in *Clostridioides difficile*

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Clostridioides difficile is a leading cause of hospital-acquired diarrhea, and vancomycin is the current first-line recommended treatment. Reduced susceptibility, however, is more frequently observed in epidemic lineages such as RT027 than in non-epidemic strains. Vancomycin resistance is primarily driven by mutations in the *vanR/vanS* regulatory system of the *vanG* operon, which has been reported to be not universally conserved in *C. difficile*. Here, we investigated the population-wide distribution and evolutionary context of the *vanG* operon and its implications for vancomycin resistance.

We analyzed >27,000 publicly available genomes, dereplicated into ~1,400 representative strains, and phylogenetically classified them into ten clades (C1-C5 and cryptic clades C-I-C-V). We profiled the distribution of antibiotic resistance-associated genes across all clades and performed codon usage analyses to investigate the evolutionary origin of the operon.

Our results revealed that resistance determinants for metronidazole (*nimB*), fidaxomicin (*rpoB*, *rpoC*), and fluoroquinolones (*gyrA*) were nearly ubiquitous (97.7-100%) across clades. In contrast, the *vanG* operon, consisting of *vanR*, *vanS*, *vanG*, *vanXY*, and v*anT*, showed strong clade-specific variation, with high prevalence in C1, C2, and C-V (74.2-100%), intermediate levels in C-I and C-II (42.9-61.9%), and low or completely absent in C3, C4, C5, C-III, and C-IV (0-5.9%). Among 837 previously published genomes carrying *vanR/vanS* resistance-associated mutations, 88.5% belonged to C2 and 9.9% to C1, with none belonging to other clades. Codon usage analysis revealed that the *vanG* operon differs markedly from housekeeping genes, clustering closer to Wright's expected curve, supporting a non-native origin.

Overall, both the distribution of the *vanG* operon and vancomycin resistance are lineage-dependent, suggesting that their emergence and clinical impact in *C. difficile* are phylogenetically constrained.

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Repurposing Metformin for Preventing Recurrent *Clostridioides difficile* Infection

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Clostridioides difficile infection (CDI), characterized by high morbidity, mortality, recurrence, and rising antibiotic resistance, remains an urgent public health threat. Effective non-antibiotic therapies are critically needed for both primary and recurrent CDI (rCDI). Metformin, a widely prescribed antidiabetic drug, has emerging immunomodulatory and microbiome-modulating properties. We previously identified metformin as protective against CDI, and here we show that it also markedly ameliorates rCDI. In a mouse model, metformin significantly reduced disease severity, minimized body weight loss, and achieved 100% survival compared with 50% in controls. Metformin-treated mice exhibited decreased fecal *C. difficile* burden and toxin levels, along with reduced gut permeability, as indicated by lower serum LBP and diminished intestinal IL-6, IL-1β, and TNF-α expression. Histological analyses revealed preserved mucus layers, intact villi, and increased expression of tight junction proteins Occludin and ZO-1, indicating strengthened barrier integrity. While microbiome changes were modest on day 10 post-infection, three days after vancomycin-induced recurrence, more pronounced differences were detected on day 2 post-infection, prior to vancomycin treatment. These findings demonstrate that metformin protects against rCDI by reducing pathogen burden, reinforcing the intestinal barrier, suppressing inflammation, and potentially modulating the gut microbiome, supporting its promise as a novel therapeutic strategy.

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Friday, July 10

Friday Poster Abstracts

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Friday, July 10

Friday Poster Abstracts *(CONT.)*

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Friday, July 10

Friday Poster Abstracts *(CONT.)*

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Development of a Molecular Assay to Quantify Butyrate Synthesis Potential in Clinical Samples

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Introduction: Butyrate is a bioactive short-chain fatty acid produced by anaerobic gut commensals within the Bacillota phylum. Butyrate deficiency is associated with loss of colonization resistance and dysregulated immune and metabolic function. Direct measurement of butyrate, however, is resource intensive, limiting its utility in large-scale studies. Consequently, a major gap in the field is the lack of molecular approaches to estimate microbial butyrate synthesis potential. The but gene is a master regulator of the microbial butyrate synthesis, but substantial phylogenetic heterogeneity complicates its quantification. This study aimed to develop and validate a molecular assay to quantify phylogenetically diverse but genes in clinical samples.

Methods: Primer sets targeting phylogenetically distinct but gene clusters (butA, butB, butC, butD, butE) were obtained. Representative butyrate-producing bacterial strains were used to validate target detection in pure culture using qPCR. The assay was subsequently applied to murine and human stool samples at baseline and following antibiotic exposure. Amplicon identity was confirmed by Sanger sequencing.

Results: Target gene identity was confirmed by Sanger sequencing and qPCR reliably detected but genes in pure cultures, with robust amplification of clusters A and D. In murine stool, butB, butC, and butD were detected at baseline and after antibiotic exposure. Notably, but gene prevalence varied by antibiotic class, supporting its potential utility as a molecular marker of antibiotic-induced gut microbiome perturbation. In stool samples from healthy human volunteers, butA, butB, butC, and butD were consistently detected, demonstrating assay applicability to human clinical specimens.

Conclusions: We developed and validated a gene-targeted qPCR assay capable of detecting phylogenetically diverse but genes in murine and human stool. This approach provides a feasible molecular tool to estimate microbial butyrate synthesis potential and to assess perturbation in gut microbiome function

Funding: NIH NIAID R01AI139261

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PGPP Post-Translational Regulation of P-Cresol Metabolism

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Clostridioides difficile (C. difficile) is an anaerobic, spore and biofilm forming bacterium causing disease ranging from antibiotic-associated diarrhea to severe colitis, typically following disruption of the normal microbiome after antibiotic use. *C. difficile* is a poor competitor against the natural microbiome in the human gut. To combat this, *C. difficile* is able to metabolize tyrosine and para-Hydroxyphenylacetic acid (*p*-HPA) to produce para-cresol (p-cresol). P-cresol is a toxic compound that inhibits growth of Gram-negative bacteria. Through the production and secretion of p-cresol, *C. difficile* is able to disrupt other native bacteria allowing it to colonize and infect the human gut. Despite this, when exposed to sublethal concentrations of p-HPA and p-cresol, the normal growth pattern of *C. difficile* is disrupted and the levels of p-Cresol produced in a *relQ* deletion strain drastically decrease. This suggests that the regulation of this pathway is necessary to ensure survival while maintaining an advantage in colonization. The ABC transporter TyrAP imports tyrosine and p-HPA, and the HpdBCA complex converts p-HPA to p-cresol. Regulation of TyrAP is thought to involve the alarmone pGpp, synthesized by the small alarmone synthetase RelQ. Metabolomic analysis of a relQ deletion strain showed that intracellular levels of tyrosine were decreased, but the levels of other tyrosine derivatives remained unchanged. This suggests that *C. difficile* tyrosine import is disrupted in the absence of alarmone pGpp, but it is able to maintain tyrosine derivative levels either by self synthesis of tyrosine or by re-routing tyrosine from other metabolic processes to maintain these levels. Together, these findings give an insight into the regulatory role of the alarmone pGpp in regulating the p-cresol metabolic pathway and a novel post-translational regulatory role of pGpp that has not yet been reported in *C. difficile*.

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Ecological Shifts in the Distribution of Anaerobic Taxa in Raw Sheep Milk Following Mastitis Therapy

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Background: Raw ovine milk is extensively used for dairy production in Mediterranean countries and, under specific hygiene and regulatory requirements, is permitted for processing within the European Union. Clinical mastitis and intramammary antimicrobial therapy can disrupt the mammary gland microbial ecosystem, potentially modifying both technological quality and the microbiological risk profile of raw milk at the point it re-enters the food chain. This study investigated therapy-associated ecological shifts in Bifidobacterium spp., Clostridium perfringens (vegetative and spore forms), and Bacillus spp. in raw sheep milk.

Methods: Raw milk samples were collected from 75 sheep farms in Greece. Animals were categorized as clinically healthy controls (Group A) or clinical mastitis cases sampled before treatment (Group B). Mastitis animals were treated with penicillin-streptomycin (B1→C1), tetracycline (B2→C2), or enrofloxacin (B3→C3). Post-therapy samples were collected on the first day after completion of the legally required withdrawal period (Groups C1-C3). Target organisms were quantified/detected using culture-based methods; C. perfringens vegetative cells and spores were differentiated using standard spore-recovery procedures prior to culture. Differences in isolation frequencies were assessed using χ^2 tests ($p<0.05$).

Results: Bacillus spp. prevalence differed significantly by regimen: 28% (A), 34% (B1) to 28% (C1); 26% (B2) to 38% (C2); and 6% (B3) to 8% (C3) ($p<0.05$). Vegetative C. perfringens increased from 13% (A) to 25-36% (B groups) and

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(CONT.)

then changed post-therapy to 35% (C1), 38% (C2), and 10% (C3) ($p<0.05$). Spore forms decreased in mastitis (49%, 36%, 36% in B1–B3 vs 62% in A) but increased after withdrawal, especially in C2 (72%) and C3 (74%) ($p<0.05$). Bifidobacterium abundance was highest in A (2.59%), dropped in mastitis (0–0.05%), and partially recovered in C1 (1.49%) and C3 (0.92%) but not C2 (0%).

Conclusions: Mastitis therapy induced regimen-specific restructuring of raw ovine milk microbiota. Beyond the post-withdrawal enrichment of C. perfringens spores—particularly after tetracycline and enrofloxacin—marked shifts were also observed in Bacillus spp. prevalence and in the recovery of Bifidobacterium spp., which was highest in healthy animals, suppressed during clinical mastitis, and only partially restored after penicillin-streptomycin and enrofloxacin but remained undetectable after tetracycline, underscoring both food-safety and quality-relevant impacts at the point of market eligibility.

Functional Diversity Across *Akkermansia* Clades Reveals Genomic and Metabolic Features Associated with Protection in the Mouse Model of MS

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system that is influenced by environmental factors, including the gut microbiome. *Akkermansia* muciniphila is associated with beneficial clinical outcomes in MS and ameliorates disease in the mouse model of MS, experimental autoimmune encephalomyelitis (EAE). However, the microbial factors underlying these effects are poorly understood. To investigate mechanisms of *Akkermansia*-mediated protection, we previously compared multiple *Akkermansia* isolates representing distinct phylogenetic clades for their ability to modify EAE severity. Oral administration of individual isolates resulted in different effects on disease, with *Akkermansia massiliensis* BWH-H3 providing the greatest protection. We therefore sought to identify genomic and metabolic features associated with this enhanced protective phenotype.

Untargeted metabolomic profiling of bacterial cultures demonstrated distinct metabolic signatures between protective and less protective isolates, including alterations in methionine cycle metabolites. To identify genomic features that could underlie these metabolic differences, we performed comparative genome analysis and found that the highly protective *A. massiliensis* strain encoded a complete cobalamin (vitamin B12) biosynthetic pathway that was absent from less protective isolates. Given the central role of cobalamin in methionine metabolism, this pathway may contribute to the observed metabolic differences between strains. Similar alterations in methionine-associated metabolites were observed in serum of *Akkermansia* colonized mice, suggesting that bacterial metabolic differences are reflected in the host environment. Together, these findings identify cobalamin biosynthesis and associated methionine metabolism as candidate mechanisms underlying the enhanced protective effects of *A. massiliensis* in EAE. This work highlights functional diversity within the *Akkermansia* genus and suggests that species- and strain-level variation may be determinants of microbiome-mediated regulation of MS.

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Deciphering the Role of TCA Cycle Enzymes in the Virulence of *Clostridioides difficile*

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C. difficile infection (CDI) is estimated to cause nearly half a million infections with approximately 30,000 deaths/year in the United States alone. Increasing resistance to first-choice antibiotics, together with recent clinical trials failures for new antibiotic drug candidates underscores the need for alternative therapeutic strategies. The major virulence factors of *C. difficile*, TcdA and TcdB, are known to be metabolically regulated. Here, we sought to define antivirulence targets by focusing on the partial TCA cycle in *C. difficile*, which contributes to energy production and supplies intermediates for different metabolic pathways. In a previous study, we showed that deletion of *feoB1* reduced TcdA/TcdB production, correlating with downregulation of genes in the oxidative branch of the TCA cycle. Given that pyruvate accumulation suppresses toxin production, we focused on the oxidative branch of the TCA cycle by creating gene knockouts in glutamate dehydrogenase (GluD), which converts L-glutamate into 2-oxoglutarate, and Pyruvate carboxylase (Pyc) that converts pyruvate to oxaloacetate. Deletion of *gluD* resulted in significantly reduced toxin production and impaired growth, whereas pyc deletion mutants had not observed effects. We speculate that in *pyc* mutants, pyruvate may be redirected to other metabolic pathways, avoiding its build-up and effects on growth and toxin biosynthesis. Conversely, the loss of *gluD* may have affected Stickland metabolism, a critical pathway for amino acids fermentation, energy generation, and maintenance of NAD/NADH redox balance. Ongoing studies aim to elucidate the mechanistic basis of reduced toxin production in *GluD* mutants and define whether this protein is a potential antivirulence drug target using both *in vitro* and *in vivo* studies.

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FABK as Narrow Spectrum Drug Target in Gram-Negative Periodontal Pathogens

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Periodontal disease (PD), an infection affecting ~47% of adults >30 years, involves bacterial induced inflammation that can lead to alveolar bone resorption and tooth loss. *Porphyromonas gingivalis* (Pg) acts as a keystone pathogen in PD and its establishment in the subgingival community is aided by binding to *Fusobacterium nucleatum (Fn)* surface proteins. To address the need for technologies that eliminate *Pg* and/or *Fn* from PD with disrupting health oral microbiota, we focused on the de novo fatty acid synthesis pathway in *Pg* and *Fn*. Bacterial fatty acids are made via specific FAS-II enzymes that are structurally different to the mammalian multifunctional fatty acid synthase. Thus, FAS-II is attractive for antibacterial discovery, particularly enoyl ACP-reductases (ENRs). Among the four main bacterial ENRs (FabI, FabK, FabL & FabV), FabK is the only nitronate monooxygenase flavoenzyme that uses FMN and NAD(P)H cofactors to perform nucleophilic enoyl reductions. *Pg* and *Fn* encode FabK as their sole ENR. Since the structural and mechanistic uniqueness of FabKs may enable the design of narrow-spectrum inhibitors, we investigated this concept in *Pg* and *Fn*. We screened a panel of FabK inhibitors with inhibitory activity against *Pg* and *Fn*. Supplementation with exogenous fatty acids did not bypass antibacterial activity against *Pg* and *Fn*. However, fatty acids rescued the growth of Streptococcal species that also carried FabK. The compounds exhibited bactericidal activity, with measurable minimum bactericidal concentrations (MBCs) against the Pg strain ATCC 33277 and Fn strain ATCC 25586. To further determine the essentiality of FabK in *Pg*, ongoing studies are genetically manipulating this organism, while the efficacy of compounds in rodent PD model will be explored.

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Narrow-Spectrum Antibacterial Therapeutics that Selectively Target *Clostridioides difficile*

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Clostridioides difficile infection (CDI) is a leading cause of antibiotic-associated infections, morbidity, and mortality in healthcare settings. Recurrence following treatment with vancomycin or metronidazole occurs in over 20% of patients and may be driven by persistent dysbiosis of the gut microbiota. Resistance to these agents is also now common and contributes to therapeutic failure. Although the narrow-spectrum antibiotic fidaxomicin (FDX) is associated with a reduced risk of recurrent CDI (rCDI), resistance has already emerged, highlighting the need for novel narrow-spectrum therapeutics. We previously validated the *C. difficile* enoyl ACP-reductase FabK as a drug target and demonstrated that a first-generation lead compound (296), belonging to the pyridinyl-thiazole-imidazole (PTIM) class, exhibited narrow-spectrum activity, non-absorption from the large intestine, and efficacy in mice with CDI. To advance PTIMs toward drug lead candidates, we conducted structure-activity relationship (SAR) studies incorporating SAR-by-catalogue screening, chemical synthesis and expansion, enzymatic assays, and pre-clinical microbiological assessment. These efforts led to the discovery of second-generation inhibitors. Analysis of >100 molecules identified a second-generation PTIM series leading to four lead candidates with minimum inhibitory concentrations (MICs) ranging from 0.1 to 1.6 µg/mL against C. difficile R20291. Using isogenic paired *Enterococcus faecalis* strains expressing either FabK or FabI, PTIMs selectively inhibited only the FabK-expressing strain and lacked activity against *Bacteroides spp*. Collectively, these findings position PTIMs for advanced pre-clinical in vitro and in vivo mechanistic and drug evaluation studies, including pharmacokinetic/pharmacodynamic analysis, resistance profiling, spectrum-of-activity using MIC testing against gut microbiota and murine microbiome models, and efficacy tests in animal models of CDI.

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Cell Senescence Following *Clostridioides difficile* Infection in Mice: Potential Signaling Targets in Enteric Glia

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Post-infection functional bowel disorder is a major complication of *Clostridioides difficile (C. difficile)* infection (CDI). *C. difficile toxins* A (TcdA) and B (TcdB) are the primary virulence factors in CDI. Senescence in enteric glia that survived TcdB exposure has previously been detected in vitro. Senescence is characterized by irreversible cell-cycle arrest, metabolic changes, and DNA damage. Although P2X purinoceptor 7 (P2X7R), adenosine receptor A2B (A2BR), toll-like receptor 4 (TLR4), transient receptor potential vanilloid 4 (TRPV4), and S100 calcium-binding protein B/receptor for advanced glycation end products (S100B/RAGE) signaling have been shown to modulate proinflammatory responses and/or cell death in enteric glia exposed to *C. difficile* toxins, it is unknown whether these pathways contribute to senescence induced by *C. difficile* toxins. This study aimed to investigate the senescence response in colonic tissues after CDI and enteric glia after TcdA/B exposure. 8-week-old mice were infected with *C. difficile* (VPI10463), monitored daily, and euthanized on days 3 and 21 post-infection to assess the senescence marker p16 by immunofluorescence in colonic tissues. In vitro, wild-type (WT) or S100BKO enteric glia cells were incubated with TcdA (50 ng/mL) or TcdB (1 ng/mL) for 18 h, followed by medium replacement without toxins/drugs every other day. At 96 h post-toxin challenge, senescence-associated β-galactosidase (SA-βGal) activity was measured. To evaluate the contribution of P2X7R, A2BR, TLR4, TRPV4, and S100B/RAGE signaling to *C. difficile* toxin-induced senescence in enteric glia cells, A438079 (300 μM), ATL801 (100 μM), C34 (50 μM), RN-1734 (100 μM), and FPSZM1 (30 μM) were added 1 h prior to toxin challenge, respectively. In vivo studies revealed increased p16, a senescence marker, in the mucosa/submucosa ($p = 0.002$) and muscular colonic ($p = 0.003$) layers of C. difficile-infected mice compared with uninfected mice on day 21 post-infection, but not on day 3 post-infection. In vitro, TcdA and TcdB increased SA-βGal activity in wild-type enteric glial cells. Blockade of P2X7R, A2BR, RAGE, or TLR4, but not TRPV4, significantly decreased SA-βGal activity in enteric glia exposed to either toxin. S100B deletion also decreased senescence-like phenotype in enteric glia exposed to TcdA or TcdB. Our findings demonstrate senescence in

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(CONT.)

colonic tissues during the recovery phase of CDI in the mouse model. In addition, in enteric glia in vitro, senescence after toxin exposure can be prevented by modulating P2X7, A2BR, S100B/RAGE, or TLR4 signaling. More studies are needed to better understand the interplay between cell senescence and gastrointestinal dysfunction post-infection, and to identify targets to prevent damage to the enteric nervous system.

Molecular Detection and Phylogenetic Characterization of *Clostridium perfringens* and Related Uncultured Lingeages from Swine in Southwestern Nigeria

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Clostridium perfringens is an important anaerobic pathogen in both veterinary and human medicine and is commonly associated with enteric disease and foodborne infections. Despite its relevance to animal health and zoonotic transmission, its molecular diversity within swine production systems in sub-Saharan Africa remains poorly characterized. This study investigated the prevalence and phylogenetic diversity of *Clostridium perfringens* and related clostridial lineages in commercial pig farms in southwestern Nigeria.

A total of 106 faecal samples were collected from three farms and analyzed using anaerobic culture followed by molecular confirmation. Sixty-five isolates (61.3%) were presumptively identified as *Clostridium* species based on characteristic colony morphology and Gram-positive rod staining. Genomic DNA extracted directly from faecal samples was subjected to conventional polymerase chain reaction targeting the *Clostridium*-specific 16S ribosomal ribonucleic acid gene and the triose phosphate isomerase (*tpi*) housekeeping gene. Twenty-one samples (32.8%) were molecularly confirmed as *Clostridium* species, with higher detection in piglets than in adult swine, although differences were not statistically significant (p = 0.689).

Bidirectional sequencing of 16S ribosomal ribonucleic acid amplicons revealed 85–95% similarity to *Clostridium perfringens* strain 4928STDY7387880 and multiple uncultured *Clostridium* clones in GenBank. Phylogenetic analysis demonstrated the coexistence of established *C. perfringens* strains and previously unclassified clostridial lineages within farm environments.

The detection of diverse and partially characterized clostridial populations in swine highlights the potential role of pig farms as reservoirs of anaerobic pathogens with veterinary and public health implications. These findings underscore the importance of integrating molecular surveillance into routine veterinary monitoring to strengthen biosecurity, food safety, and zoonotic risk prevention strategies.

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The Effect of Biochanin A on the Antibiotic Susceptibilities of *Escherichia Coli* Grown Under the Anaerobic Fermentation Versus Oxygenic Respiration and Differing Metabolic conditions on Agar Media

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Antimicrobials have been used for a number of purposes in beef cattle production. However, increased prevalence of pathogens, including zoonotic bacterial species, such as *Escherichia coli*, is a concern. The isoflavone, biochanin A (BCA), was identified as a natural feed component to potentially limit the spread of antibiotic-resistant microorganisms by potentiating the activity of other antimicrobials. The current study investigated the synergistic effects of 120 ppm BCA with five classes of antibiotics on two strains of *E. coli*: 25922 and 51659 (O157:H7) on two agar culture media with commercial ETEST® strips and the FDA/CLSI breakpoint interpretation criteria. The effect of 120 ppm BCA on minimum inhibitory concentration (MIC) across the antibiotic classes was relatively consistent where it had largely no effect, an occasionally negative effect, or a neutral effect. BCA addition yielded negligible changes to ciprofloxacin (susceptible) for either strain under the redox and metabolic conditions tested. The culture conditions seemed more influential over MIC than BCA. The absence of oxygen generally conferred clinical resistance or a higher MIC, and at times media composition altered the MICs as well. For example, neither macrolide inhibited either strain (> 256 μg mL^{-1}) under anaerobic conditions but generally had measurable MICs aerobically. Additionally, the MICs for streptomycin were consistently higher across each strain when cultured on tryptic soy agar rather than Mueller Hinton broth. Despite previous studies in which biochanin A decreased MICs in other bacterial species, neither *E. coli* strain was consistently impacted by the presence of 120 ppm BCA in the culture medium. The data indicate that BCA is likely not a reliable resensitizing/synergistic candidate for resistant *E. coli*. Regardless, the data demonstrates the impact culture conditions, including the presence or absence of oxygen, have on MIC across antibiotic class. These differences in susceptibility may be necessary to consider when treating *E. coli* infections under different conditions.

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Microbiome Restoration Potential of Ibezapolstat vs. Comparator Antibiotics in Patients with Multiply Recurrent *Clostridioides difficile* Infection (CDI)

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Background: Narrow spectrum antibiotics in development (ibezapolstat; IBZ) have promising attributes that could reduce CDI recurrence vs. vancomycin (VAN) due to a narrower disruption of the healthy microbiome. In phase 1-2 IBZ trials, regrowth of beneficial microbes of the Actinomycetota phylum as well as certain Bacillota families (Lachnospiraceae and Oscillospiraceae) were observed. Microbial diversity decreases with increased abundance of Enterococcus in patients with multiply recurrent CDI but continued utility of narrow spectrum antibiotics in this patient population has not been fully studied. The purpose of this study was to assess microbiome changes in CDI patients based on number of CDI recurrence and to study the in vitro effect of IBZ and VAN on C. difficile and Enterococcus growth.

Methods: Metagenomic evaluations were assessed using stool samples from healthy volunteers (n=19), and hospitalized patients with antibiotic associated diarrhea without CDI (AAD; n=26), primary CDI (n=110), or recurrent CDI (n=51). In separate studies, IBZ and VAN were tested in co-culture models to assess killing of C. difficile and Enterococcus in mono- vs. duo-culture studies.

Results: Healthy volunteers had a high proportion of beneficial commensals including bacterial species from Actinomycetota and Bacteroidota phylums and Lachnospiraceae and Oscillospiraceae families. Proportion of these beneficial commensals decreased in patients with AAD and CDI but were still present. Proportions decreased in patients with recurrent CDI but were still present at lower abundance. Recurrent CDI patients had a higher abundance of Enterocaccaceae, specifically vancomycin-resistant Enterococcus (VRE). In co-culture models, VAN caused overgrowth of VRE which did not occur with IBZ.

Conclusion: Continued preservation or regrowth of beneficial commensals is likely to be an important consideration in determining antibiotic choice for patients with primary or recurrent CDI. Future clinical trials are necessary to determine the effects of beneficial commensal regrowth on treatment outcomes for VAN and IBZ.

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Successful Fecal Microbiota Transplants in Post-Antibiotic Recurrent *Clostridioides difficile* Patients Induce Lipidomic Shifts

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Clostridioides difficile infection (CDI) is a major global health threat, with ~30% of patients experiencing recurrence post-antibiotics. Current recurrence prevention strategies, including microbiota-focused therapeutics and fecal microbiota transplants (FMTs), face safety, availability, and standardization challenges, driving demand for live biotherapeutics with defined mechanisms. To better characterize such mechanisms in FMTs, we longitudinally profiled lipids from patient stool using a liquid chromatography, ion mobility spectrometry, and mass spectrometry platform. We find that FMT significantly reshapes the lipidome (PERMANOVA R^2=0.12, $p<0.001$), altering 96 species across 18 classes. The most differentially abundant lipids were acylcarnitines, which decreased post-FMT. Medium- and long-chain acylcarnitines also strongly co-occurred with Enterobacteriaceae, species of which have been previously demonstrated to utilize carnitine for growth in IBD patients. Additionally, we found that Enterobacteriaceae drove pre-FMT increases in amino acid biosynthesis pathways essential for *C. difficile* growth (e.g., cysteine, isoleucine), leading to the postulation of a cross-feeding hypothesis. Interestingly, we found that Lachnospiraceae including *Blautia* replaced Enterobacteriaceae post-FMT and contributed significantly to amino acid biosynthesis capacity after treatment. These findings highlight lipids as important metabolic factors in patient susceptibility to rCDI and suggest the interactions between microbiota and lipids pre- and post-FMT as targets for developing next-generation live biotherapeutic products.

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Secondary Microbially Conjugated Bile Acids Differentially Affect Different Stages of the *Clostridioides difficile* Lifecycle

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Clostridioides difficile is a Gram-positive, spore forming bacterium responsible for *C. difficile* infection (CDI) and ~29,000 U.S. deaths per year. The gut microbiota provides colonization resistance through nutrient competition and producing inhibitory secondary bile acids. A critical step of secondary bile acid production is the removal of host conjugated amino acids (AAs) by bacterial bile salt hydrolases, which have recently been shown to reconjugate amino acids to free bile acids to create microbially conjugated bile acids (MCBAs). There has been an association between secondary MCBAs and successful fecal microbiota transplants in patients with recurrent CDI, suggesting a role for secondary MCBAs in colonization resistance. We hypothesize that secondary MCBAs with deoxycholate (DCA) or lithocholate (LCA) sterol cores have variable effects on different stages of the *C. difficile* lifecycle compared to their unconjugated counterparts. To address this, we synthesized secondary MCBAs with nine amino acids (Cys, Iso, Leu, Phe, Pro, Ser, Trp, Tyr, Val) and tested their impact on *C. difficile* growth and membrane integrity via 24 hr growth assays in MCBAs and propidium iodide staining, respectively. We show that modification of the sterol core significantly alters the effect on *C. difficile* growth. AA-DCAs like Ile-, Leu-, and Trp-DCA are more inhibitory while Cys-, Pro-, and Val-DCA are less inhibitory compared to DCA. Membrane integrity assays show Ile- and Pro-DCA reduced membrane disruption compared to DCA. Cys- and Tyr-LCA did not inhibit growth at LCA's MIC of 1.2 mM and Val-LCA was inhibitory to growth at concentrations well below the MIC of LCA at 0.0375mM. Ile-, Pro-, and Val-LCA reduced membrane disruption compared to LCA. This data suggests that these MCBAs may be potent inhibitors of *C. difficile* but do so through a different mechanism than membrane disruption. Future experiments testing toxin expression and sporulation are planned with an expanded library of MCBAs.

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A Lipid Biosynthetic Gene Cluster in *Parabacteroides* Confers Tolerance to Bile Acids

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The order Bacteroidales is among the most abundant group of bacteria in the human gut. Despite their importance roles in human health, the mechanisms by which Bacteroidales thrive within the human gut remain poorly characterized. In recent years, high-throughput functional genomics methods such as Barcoded Overexpression BActerial shotgun library sequencing (Boba-seq) have revealed genetic factors important for stress tolerance. In Boba-seq, we use DNA barcode sequencing to quantify strain abundances in competitive fitness assays of diverse genome-wide libraries, which uncovered new genes important for tolerance to antimicrobials, including bile acids. Bile acids are host-produced compounds, present in the intestines, and aid in food digestion. They also have antimicrobial properties that shape the microbiota composition. We identified a lipid biosynthetic gene cluster conserved in *Parabacteroides* and absent in *Bacteroides*, two prominent genera in the gut. We observe increased tolerance towards certain bile acids when this cluster is overexpressed in *Bacteroides*. Conversely, increased susceptibility to primary and secondary bile acids is observed in *Parabacteroides* deletion mutants. The genes are part of protein families involved in lipid biosynthesis, but the encoded enzymes have not been characterized. From lipidomics data, total lipid compositions of wild-type strain and cluster deletion mutant are significantly different. Functional studies of these genes will allow us to identify lipid metabolites and their role in bile acid tolerance. Our work uncovered a novel, conserved pathway in *Parabacteroides* species important for bile acid tolerance and advances our understanding or how anaerobes tolerate antimicrobial stress in the gut.

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A Report on Increasing Carbapenem Resistance Among *Bacteroides* Strains in Hungary

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Statement of Purpose: Carbapenems are wide-spectrum and reserve antibiotics treating bacterial infections. Bacteroides show low resistance rates to them but carbapenem resistance can occur and aggravate therapy. The best known resistance mechanisms against cabapenems among *Bacteroides* strains are the species-specific cfiA (*B. fragilis*) and crxA (*B. xylanisolvens*) genes activated by insertion sequence (IS) elements giving rise ca. 1 % resistance rates.

Methods: Carbapenem resistant strains were isolated in clinical diagnostics during 2025 by the EUCAST disc diffusion method and after this MICs for a panel of antibiotics, including carbapenems, were determined by a gradient method (Etest). Resistance genes and upstream IS elements were detected by PCR and the collected strains were subjected to whole genome sequencing.

Results: Out of 341 *B. fragilis* and 10 *B. xylanisolvens* isolated strains 4 (1.2%) and 1 (10%) resistant isolate were included for further characterization. These numbers mean an increasing prevalence since only rare sporadic cases were found previously in Hungary. All 4 *B. fragilis* strains were cfiA and IS element-positive with varying activating IS types (IS1169, IS613, IS1380-like). The *B. xylanisolvens* strain was *crxA*-positive. Other antibiotic, including metronidazole, Etests results and the analysis of genomes showed that the strains were not multidrug-resistant.

Conclusions: We can expect a deteriorating carbapenem resistance situation among *Bacteroides* strains in Hungary that we expect to be caused by insertion of various IS elements to the upstream regions of the resistance genes and is not caused by clonal strains.

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Antimicrobial Activity of Viable and Heat-Killed Wild-Type Lactic Acid Bacteria Isolated from Fermented Foods

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Foodborne pathogens, such as *Salmonella*, *Staphylococcus*, and *Escherichia spp.* are a major cause of considerable economic losses, illnesses, and mortality worldwide. The rising prevalence of antimicrobial resistance among these pathogens has intensified efforts to identify alternative antimicrobial agents, including non-viable microorganisms and microbial cell-derived extracts. In this study, 14 wild-type lactic acid bacteria belonging to *Lactiplantibacillus plantarum*, *Lactiplantibacillus herbarum*, *Lentilactobacillus buncheri*, *Lacticaseibacillus paracasei*, *Pediococcus acidilactici*, and *Leuconostoc lactis* species, were isolated from fermented foods, such as olives, kefir, and pickled peppers and their growth-inhibitory activity against *Salmonella* Enteritidis, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* was evaluated before and after heat-treatment at 80 °C and 120 °C. Initial screening using the well-diffusion assay identified 6 strains, belonging to L. plantarum and *L. paracasei* species, with inhibitory zones ranging from 8.00 to 21.00 mm after heat-treatment. All selected strains demonstrated strong aggregation rates (over 73.25%) with all tested pathogens after 24 h of incubation, a property maintained after heat treatment at both temperatures. In the co-culture assay, viable cells significantly reduced pathogen growth, with the most pronounced effect observed against *S. aureus*; all strains reduced bacterial counts from 8.60 logcfu/mL to below 5.80 logcfu/mL. In contrast, no significant reduction in pathogen growth was observed when heat-killed cells were used. Furthermore, the minimum inhibitory concentration (MIC) of freeze-dried cell-free supernatants (LCFS) was determined. MIC values ranged from 8.00 to 32.00 mg/mL; *L. plantarum* EP1 strain exhibited the lowest MIC value (8.00 mg/mL) against all pathogens, even after heat-treatment. These findings highlight the antimicrobial potential of both viable and heat-killed lactic acid bacteria and support further investigation as alternative agents against foodborne pathogens.

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Nanoparticle-Delivered MIRNA-23A-3P and MIRNA-150-5P: Evaluating Barrier Protection and Anti-*Clostridioides difficile* Activity Across Clinical Strains

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Patients with inflammatory bowel disease (IBD) are at increased risk of *Clostridioides difficile* infection (CDI), which can trigger severe flares and hospitalisation. MicroRNAs (miRNA)-23a-3p and miRNA-150-5p are downregulated in CDI/colitis models and have been implicated in cytoprotective responses to *C. difficile* toxins via pathways linked to epithelial junction integrity. While this supports miRNA replacement as a host-directed strategy, it remains unclear whether therapeutic miRNAs (and their delivery vehicles) exert direct effects on *C. difficile* biology.

Here we evaluate a range of lipid- and polymer-based nanoparticles co-delivering miRNA-23a-3p and miRNA-150-5p with a dual focus on epithelial barrier protection and direct activity across a panel of clinically relevant *C. difficile* strains. Barrier protection was assessed in Caco-2/TC-7 monolayers by transepithelial electrical resistance (TEER) and fluorescein-dextran permeability following challenge with purified toxins TcdA/TcdB. Direct antibacterial effects are being quantified using MIC testing, sporulation and germination assays, and toxin quantification by ELISA. To probe mechanism, fluorescently labelled miRNA/nanoparticles are tracked by confocal microscopy to assess binding and internalisation into bacterial cells.

Preliminary data show efficient epithelial uptake into Caco-2/TC-7 cells without detectable cytotoxicity, and combinatorial (but not single-miRNA) delivery preserves TEER and reduces permeability after toxin challenge ($p<0.05$). Ongoing work will determine whether miRNA delivery modulates *C. difficile* viability, spore and toxin production, and whether internalisation into bacterial cells correlates with functional effects. Future characterisation will employ 3D OrbiSIMS and transcriptomics to map nanoparticle/miRNA localisation and the associated host-pathogen molecular responses *in situ*.

Presented by **SARAH KUEHNE**

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Further Understanding the Role of a 7A-Dehydroxylation in Resistance Against *Clostridioides difficile*

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A healthy microbiome provides resistance against *Clostridioides difficile* infections (CDI) through several mechanisms, including the metabolism of primary bile acids. Antibiotic use disrupts the gut microbiome and reduces species capable of performing 7α-dehydroxylation, the conversion of primary bile acids, which stimulate *C. difficile* spore germination, into secondary bile acids, which inhibit germination, outgrowth, vegetative growth and toxin activity. Healthy individuals consistently exhibit higher secondary bile acid levels than CDI patients. Loss of this metabolic function is therefore hypothesised to contribute to CDI susceptibility.

This study combines mechanistic and synthetic approaches to further characterise the 7α-dehydroxylation pathway and explore strategies to restore bile-acid-mediated colonisation resistance. First, we constructed a bank of *Peptacetobacter hiranonis* strains overexpressing individual genes of the bile acid-induced (*bai*) operon. Using plasmid-based expression under the native PsecG promoter, all eight *bai* genes were successfully introduced and verified via colony PCR, sequencing, and RT-qPCR. Liquid chromatography-mass spectrometry was used to quantify bile acid derivatives, focusing on cholic acid to deoxycholic acid conversion to identify potential rate-limiting steps within the pathway.

In parallel, we engineered *Clostridium butyricum*, a species with a long safety record as a probiotic, to express the entire bai operon chromosomally via Allele-Coupled Exchange. This work aims to determine whether introducing 7α-dehydroxylation capacity into a safe species can enhance colonisation resistance against *C. difficile*.

Together, these complementary approaches provide insight into the function, regulation, and therapeutic potential of primary bile acid metabolism. Ongoing work includes testing the impact of engineered strains on *C. difficile* growth, viability, and germination, and assessing their potential as next-generation probiotics to prevent CDI.

Presented by **SARAH KUEHNE**

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The Oncogenic Role of Prevotella Intermedia in Oral Squamous Cell Carcinoma Progression

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Accumulating evidence indicates that the oral microbiota contributes to the development of oral squamous cell carcinoma (OSCC) through chronic inflammation and direct carcinogenic effects. *Prevotella intermedia* (Pi), an anaerobic opportunistic oral pathogen, has been shown to be associated with various oral diseases; however, the details of its role as a pro-tumorgenic bacterium remain under studied.

Herein, we demonstrate that Pi infection is positively correlated with oral cancer cell proliferation at both phenotypic and molecular levels. Co-incubation with Pi leads to the increased proliferation of SCC25 cells, a human OSCC cell line. We next performed whole transcriptome sequencing on human OSCC cells infected with Pi for 6 and 24 hours, along with time-matched uninfected controls (4 samples per group, total n=16). Differential expression analysis identified 731 differentially expressed genes (DEGs). At 6 hours, 393 genes were upregulated and 326 downregulated, while at 24 hours, 195 genes were upregulated and 319 downregulated.

Pathway enrichment analysis revealed a highly ordered temporal progression. At 6 hours, Pi infection triggered an acute innate immune response and metabolic mobilization, characterized by activation of HIF-1α signaling, glycolysis, and mTOR pathways, alongside downregulation of DNA repair pathways, suggesting early accumulation of genomic instability. By 24 hours, the early metabolic surge subsided and was replaced by activation of MYC and IL-6/STAT3 signaling, indicating oncogenic reprogramming and sustained proliferative signaling.

Consistent with our phenotypic findings, these results demonstrate that Pi promotes OSCC proliferation and induces coordinated transcriptomic reprogramming. Our study provides mechanistic evidence that Pi drives early inflammatory and metabolic activation followed by oncogenic pathway engagement, highlighting its potential role in shaping the tumor microenvironment and promoting oral cancer progression.

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Lachnospiraceae Bile Salt Hydrolases as Regulators of *C. difficile* Colonization Resistance and Pathogenesis

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Clostridioides difficile is a Gram-positive, spore-forming bacterium responsible for *C. difficile* infection (CDI) resulting in over 29,000 deaths annually. While CDI can be treated with antibiotics, 25-40% of patients can develop a subsequent infection, leading to recurrent CDI (rCDI). A fecal microbiota transplant (FMT) remains the only viable treatment for rCDI, though limited supply and regulatory challenges hinder its efficacy. This work aims to move beyond these untargeted therapies and leverage the innate biochemical properties of the gut microbiota to develop robust therapeutic approaches. Gut microbiota combat CDI via the synthesis and alteration of bile acids (BAs) by encoded bile salt hydrolases (BSHs). BSHs deconjugate CDI-favored primary BAs such as glycocholate (GCA) and taurocholate (TCA) into G/T and CA, which are transformed into CDI-inhibiting secondary BAs like deoxycholate (DCA). Recently, we showed that rCDI patients that underwent a FMT had high levels of Lachnospiraceae post-FMT. This shift was concurrent with reduced levels of primary BAs and increased levels of secondary BAs and *bsh* genes. Thus, we hypothesize that BSHs are required to change the gut BA pool which promotes colonization resistance against *C. difficile*. To address this, we investigated Lachnospiraceae BSHs associated with high secondary BA abundance post-FMT. Eighty-two genomes representing seven genera across the Lachnospiraceae family were isolated, whole genome sequenced and mined for BSH encoding genes. Over 200 BSH sequences were identified and clustered at 100% sequence identity, revealing 24 unique BSHs across the 82 genomes. Current efforts are underway to biochemically characterize these BSHs both *in vitro* and *in vivo* to determine their effects on *C. difficile* colonization resistance and pathogenesis.

Presented by **NOAH LYONS**

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Investigation and Characterization of *Clostridioides difficile* Infection Diagnostic Tests to Support AZD5148 Clinical Trial Readiness

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Clostridioides difficile infection (CDI) diagnosis is challenging due to the use of different diagnostic methods and variable test sensitivity and specificity. An in-depth characterization of over 200 CDI clinical samples was performed using available PCR, EIA, and CCNA diagnostics to confirm that AZD5148 does not interfere with Toxin B detection by common commercially available diagnostics.

AZD5148 is an anti-*C. difficile* Toxin B monoclonal antibody (mAb) that is currently in a Ph2b clinical trial (PRISM, NCT07285213) for the prevention of *C. difficile* infection (CDI) recurrence. CDI poses significant challenges in clinical diagnosis due to variable test sensitivity and specificity. Accurate diagnosis is crucial for effective patient enrollment and successful clinical trial outcomes. Enzyme immunoassays (EIA) are widely available, have a rapid turnaround time, and unlike PCR based tests, provide confirmation of toxin production to differentiate disease from colonization. EIA are highly specific but with a sensitivity of 60-80% may miss cases relative to the "gold" standard cell cytotoxicity neutralization assay (CCNA). CCNA boasts high specificity (98%) and sensitivity (75-90%) but is labor and time-intensive, limiting use and introducing logistical challenges in trial settings. With Phase 2b clinical trials for recurrence currently underway, these challenges must be addressed and diagnostic assay performance further characterized. Therefore, an in-depth characterization of available PCR, EIA, and CCNA diagnostics using over 200 CDI clinical samples and various purified *C. difficile* toxins was performed. Further, results showed the limit of detection of FDA approved EIAs for Toxin B ranges widely from 5 ng/mL to 200 ng/mL, evaluated by testing representative toxinotypes from the five major clades of *C. difficile*. Importantly, results confirmed that AZD5148 does not interfere with Toxin B detection by common commercially available diagnostics. Together, these data inform how CDI diagnostics can best be utilized in current and future AZD5148 clinical studies.

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Efficacy of Beta-Lactam Monotherapy vs. Clindamycin or Addition of Metronizadole in Peritonsillar Abscess--an Observational Study with a focus on *Fusobacterium necrophorum*

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Background: Peritonsillar abscesses are often caused by *Streptococcus pyogenes or Fusobacterium necrophorum*. Few studies have investigated the best choice of antibiotic therapy. In Nordic countries, penicillin is typically advocated, yet its efficacy in *F. necrophorum* infections is unknown. We aimed to compare the efficacy of beta-lactam monotherapy vs. clindamycin or beta-lactam with metronidazole in peritonsillar abscess.

Methods: This was a population-based, retrospective study on patients with peritonsillar abscess who were microbiologically investigated for β-hemolytic streptococci and *F. necrophorum* during 2013-20 in southern Sweden. Patients treated with beta-lactam (reference) were compared with those treated with clindamycin or beta-lactam with metronidazole. The primary outcome was a composite of recurrent pharyngotonsillitis or peritonsillar abscess (30 days). Logistic regression (crude and multivariable) and inverse probability weighting were performed in the full population and a *F. necrophorum*-subgroup. Multivariable analyses adjusted for hospital admission (0/1), successful drainage (0/1) and age category (0-14 years (reference), 15-40 or >40).

Results: In total, 391/899 (44%) patients with peritonsillar abscess were exposed to beta-lactam monotherapy, of which 96% received penicillin, and 498/899 (56%) to clindamycin or combination therapy with metronidazole. The primary outcome occurred in 57/899 (6%) in the entire cohort and in 39/271 (14%) in the *F. necrophorum*-subgroup. No associations between outcome and antibiotic exposure were seen in the crude analyses in the entire cohort (OR 1.4 95%CI 0.8-2.4) or the *F. necrophorum*-subgroup (OR 1.0 95%CI 0.5-2.0). Findings were similar in adjusted analyses.

Conclusions: Beta-lactam monotherapy in peritonsillar abscess, typically with penicillin, was as effective as broader antibiotic therapy, including when *F. necrophorum* was identified. Hence, it could be recommended as first-line therapy in peritonsillar abscess.

Presented by **DAVID NYGREN**

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Prevalence of *Fusobacterium necrophorum, Streptococcus pyogenes* and *Streptococcus dygalactiae* subsp. equisimilis and Accuracy of Clinical Decision Rules in Children with Pharyngotonsillitis--a Case Control Study in The Gambia

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Background: *Fusobacterium necrophorum* is a main pathogen in pharyngotonsillitis yet has never been investigated as a cause of throat infections in a low-middle income country. In high-income countries, it mainly affects adolescents and young adults and rarely children. We aimed to describe its role in children with pharyngotonsillitis in The Gambia, in addition to Streptococcus pyogenes (GAS) and S. dysgalactiae subspecies equisimilis (SDSE).

Methods: Cases with pharyngotonsillitis and controls aged 0-15 years were enrolled in Sukuta, The Gambia. Tonsillar samples were analyzed with multiplex PCR, targeting GAS, SDSE and F. necrophorum. Rates in cases and controls were compared, and accuracy of commonly used clinical decision rules, i.e., the Centor, modified Centor, FeverPAIN, Cape Town and Smeesters score, to predict F. necrophorum or any of the pathogens were evaluated.

Results: In cases (n=376) GAS was more common than in controls (n=105) (32% vs 11%, p<0.0001). F. necrophorum was equally common in cases and controls (9% vs 10%, p=0.93), as was SDSE (11% vs 14% p=0.19). Clinical decision rules had poor accuracy to identify either F. necrophorum or any of the pathogens.

Conclusions: GAS was the most common finding and the only to differ between cases and controls. Unexpectedly, F. necrophorum was more common in children with and without symptoms in The Gambia than anywhere previously described. Neither F. necrophorum or SDSE differed between cases and controls. Established clinical decision rules were not useful in this setting.

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Identification of *Enterococcus faecalis* Genes Involved in Regulation of *Clostridioides difficile* Sporulation Efficiency

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Spores are the major transmission vector of *C. difficile* between human patients and in the environment. *C. difficile* and enterococci both inhabit the intestines of humans and are frequently co-isolated from patients. Our previous work characterized a contact-dependent inhibition of *C. difficile* sporulation that required pili on the surface of *E. faecalis*. Here we report on a genetic screen in progress that uses an arrayed transposon insertion library in *E. faecalis* OG1RF to identify genes involved in the suppression of sporulation. We have identified 8 OG1RF transposon insertions that significantly enhanced C. difficile sporulation efficiency when in co-culture. All 8 loci are predicted to be present at the cell membrane, displayed on the cell wall or to be secreted. Additionally, six of the 8 identified loci had altered biofilm formation phenotypes in OG1RF. These data are consistent with a mechanism of short-range cell to cell signaling between *C. difficile* and OG1RF that may mediate sporulation efficiency. The genes identified include two predicted CHAP-domain containing peptidoglycan hydrolases. We are currently characterizing the role of these peptidoglycan hydrolases in mediating interactions between the two species.

Presented by **IBUKUN OGUNYEMI**

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Leveraging C. difficile Strain-Strain Competition to Reveal Genetic and Metabolic Factors Contribuing to colonization

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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 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, that does not cause diarrhea or morbidity in antibiotic-treated mice. We showed that ST1-75's avirulence is attributable to a 69-bp deletion in its *cdtR* gene, which reduces the expression of all three toxin genes. Interestingly, co-infection of mice with ST1-75 and virulent *C. difficile* strains prevents virulent colitis because ST1-75 rapidly outcompetes the virulent strains. To gain insight into the mechanism by which ST1-75 outcompetes R20291, we applied RNA-Seq to compare the transcriptional profiles of ST1-75 and R20291 upon murine infection. RNA-Seq analyses reveal that many amino acid metabolism pathways are highly enriched in ST1-75. Specifically, ST1-75 expresses a gene encoding a branched-chain amino acid transporter, *brnQ1*, at 40-fold higher levels than R20291. These data are consistent with our metabolomic analyses, which show that ST1-75 depletes several amino acids more rapidly, including Gly, Ala, Phe, Val, Ile, and Met. On the other hand, since R20291 produces significantly higher levels of toxins than ST1-75, which may pose a metabolic burden to R20291's competitive fitness. To test this, we generated a binary toxin knockout R20291 strain *(ΔcdtAB)* and conducted its co-infection with ST1-75. We found that ST1-75 still outcompetes R20291*ΔcdtAB*. We are currently dissecting the roles of *BrnQ* genes, CDT genes, and amino acid supplementation individually and in combination to understand their contribution to ST1-75's competitive fitness. By uncovering genetic factors and regulatory networks that distinguish high-fitness strains, this work will provide mechanistic insight into how *C. difficile* adapts to and exploits the gut environment.

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The Complex Defined Synthetic Microbial Community HCOM1 Prevents Disease from *Clostridioides difficile* Infection but not Colonization in a Gnotobiotic Mouse Model

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Clostridioides difficile infection (CDI) is a leading cause of hospital-acquired infections in the United States. In healthy individuals, the gut microbiota provides colonization resistance against *C. difficile*, whereas antibiotic treatment disrupts these protective microbial functions. While some mechanisms of protection are well described, others and their emergent interactions are poorly understood. Synthetic microbial communities provide a controlled framework to test these mechanisms by enabling targeted manipulation of community composition and function. The objective of this study was to determine whether a complex synthetic community made up of 104 human bacterial strains (hCom1) was able to prevent *C. difficile* colonization in a gnotobiotic mouse model and to identify an experimentally and biologically optimal dose. Germ-free C57BL6J mice (n=4/condition) were orally gavaged with 200 ul hCom1 (standard dose), 50 ul hCom1 (low dose), or left untreated. After seven days, all mice were challenged with *C. difficile* R20291 spores and evaluated for an additional two days. Clinical signs of disease, *C. difficile* bacterial load, toxin activity, and histopathological changes to intestinal tissue were measured. We found that only the standard dose of hCom1 conferred protection against toxin-mediated disease, with mice showing significantly less weight loss, toxin activity, and histopathological changes to intestinal tissue compared to the low dose and untreated control. However, while the hCom1 community reduced toxin mediated disease, it failed to restore colonization resistance, as *C. difficile* was still present across all conditions. Future strain tracking using metagenomics will reveal dose-dependent patterns of colonization and community assembly in both doses, and mechanistic studies will be done to determine how hCom1 is able to suppress *C. difficile* virulence.

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The Impact of Sporulation/Germination cycles in Spore-Reservoir Persistence During *Clostridioides difficile* Infection

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Clostridioides difficile recurrent infections are a major clinical challenge. *C. difficile spores* are critical for persistence of the recurrence of *C. difficile* infection (R-CDI). Our recent findings using a mouse model of R-CDI indicate that, after 15 days of vancomycin treatment, spores are still detectable in the gut epithelium, suggesting that persister cells and/or low-frequency sporulation/germination cycles maintain a persistent spore-reservoir.

We first assessed the impact of persister cells in recurrent by infecting mice with an asporogenous Δ*sigF* strain followed by vancomycin treatment at the peak of infection. The dynamics of colonization and disease severity was comparable to wild-type strain. No detectable spore-like structures were evidenced in the intestinal mucosa in colonic and cecum tissue of Δ*sigF* infected mice, correlating with absence of relapse. Next, we utilized a Δ*sleC* mutant strain, which yields a low germination frequency of germination, to infect susceptible mice. The dynamics of colonization and persistence of Δ*sleC* spores in the presence and absence of vancomycin treatment in cecum and colonic tissue were also quantified and correlated with the rate of R-CDI in Δ*sleC* infected mice. Together, results of this work contribute to understand the fundamental mechanisms implicated in persistence of *C. difficile* during antibiotic treatment and how these impact relapse of CDI.

Together, results of this work contribute to understand the fundamental mechanisms implicated in persistence of *C. difficile* during antibiotic treatment and how these impact relapse of CDI.

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Genomic Variability in Colonization Factors Across Gut Lachnospiraeceae Species

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The mammalian gastrointestinal tract harbors diverse populations of anaerobic, spore-forming bacteria within the class Clostridia. Their prevalence and consistent association with health across the human population suggests an important role in intestinal homeostasis; yet, colonization mechanisms, including germination and metabolic needs, of many Clostridial species remain poorly defined. To address this gap, we examined inter- and intra-species genomic diversity within Lachnospiraceae, a prevalent gut Clostridial family, to better define their ecological potential. From an isolation pipeline targeted at optimizing commensal Clostridia from human feces (n = 9), we focused on 21 unique Lachnospiraceae species. Colonies were initially distinguished by colony morphology, confirming identity via Sanger sequencing. We conducted whole-genome comparisons on a subset of isolates (n= 91) alongside reference genomes (n = 58) to identify shared and species-specific colonization factors, focusing on sporulation, germination, and carbohydrate use. Genomes were assembled using SPAdes, screened against a curated UniRef database of relevant genes, and annotated for carbohydrate-active enzymes (CAZymes) using dbCAN. The global regulator, *spo0A* was present across all species. Sporulation-associated sigma factors, *sigF*, *sigH*, and *sigK* were also present but varied across species, with *sigK* restricted to Anaerostipes species and sigF restricted to *Bariatricus comes* and *Dorea* species, suggesting variable sporulation pathways. Germination genes *gerAB* and *gerAC* were absent from all species, whereas *gerAA* was present in all except *Anaerostipes*. Principal Coordinate Analysis (PCoA) based on CAZyme profiles demonstrated significant clustering by species, suggesting species-specific carbohydrate utilization potential (PERMANOVA, $p<0.001$). However, intra-species variation was observed within some species including *Blautia wexlerae* and *Mediterraneibacter gnavus* (PERMANOVA, $p<0.01$). Future work will expand this analysis to additional colonization factors, including adherence mechanisms, to further understand how *Lachnospiraceae* are maintained within the gut environment.

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From Culture to Codes: Mapping the Global Landscape of AI-Driven Applications in *Clostridioides difficile* Research via Bibliometrics

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This study aimed to investigate the global scientific development and the thematic transition of Artificial Intelligence applications in *Clostridioides difficile* research.

An analysis of 515 scientific publications published up to 2025 in the Scopus database was conducted using the Bibliometrix and VOSviewer tools. The annual growth rate was 8.16%, while research production accelerated strongly after 2018 and peaked in the period 2022-2025. The time-evolution analysis reveals a clear and fundamental shift from traditional topics, such as PCR, toxin detection, stool testing, and conventional diagnosis, to modern AI-driven approaches. In recent years, terms such as machine learning, artificial intelligence, microbiome, gut microbiota, dysbiosis, antimicrobial resistance, healthcare-associated infections, surveillance and infection control have dominated. At the same time, thematic mapping showed a strengthening of fields such as faecal microbiota transplantation, precision diagnostics and predictive risk modelling, suggesting the integration of digital tools in clinical decision-making. Trend Topics analysis reveals that research is now "moving" from simple diagnosis to predictive surveillance and infection control through algorithms. The additional integration of data from electronic health records to predict relapses and the use of Deep Learning to understand antibiotic resistance are the fastest-growing fields, with the USA and China leading this technological shift.

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(CONT.)

In conclusion, research on *Clostridioides difficile* is moving from classical microbiological and diagnostic approaches to a new model based on Artificial Intelligence, the convergence of computational biology with clinical surveillance to provide personalized predictive models, microbiome analysis and risk prediction. Finally, explainable AI models and early warning systems will be further integrated into personalized prevention and treatment strategies.

A Specific Lineage of Hungatella is Associated with Progressive Multiple Sclerosis

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Purpose: Progressive multiple sclerosis (PMS) is clinically and biologically distinct from relapsing-remitting MS (RRMS) and remains largely resistant to current immunotherapy. Emerging evidence suggests that disease progression may be influenced by the gut microbiome. We sought to identify microbiome features uniquely associated with PMS using high-resolution taxonomic and functional analyses.

Methods and Results: We analyzed 16S rRNA gene V4 sequencing data at the amplicon sequence variant (ASV) level from fecal samples of MS patients in the CLIMB cohort (Boston; healthy controls [HC] n=40, RRMS n=199, PMS n=44) and validated findings in the international MS microbiome study (iMSMS) cohort (4 countries, 7 cities; HC n=576, RRMS n=437, PMS n=139). Among 3,446 ASVs, *Hungatella hathewayi* was the most prominently increased taxon in PMS. Fine-scale ASV analysis resolved two closely related *H. hathewayi* clades (A and B) sharing 99.2% 16S sequence identity. Clade B ASVs (CLIMB: ASV631; iMSMS: ASV303) were consistently enriched in untreated PMS compared with untreated RRMS and HC, whereas clade A ASVs showed no disease association. Abundance of clade B, but not clade A, correlated significantly with clinical severity in both cohorts (CLIMB: r=0.17, p=0.007; iMSMS: r=0.20, p=0.004). We next isolated patient-derived strains representing both clades and performed whole-genome sequencing. Pan-genome analysis together with public genomes (total 15 strains) revealed selective enrichment of a complete urease operon in clade B strains, which was validated by urease activity assays. Consistently, metagenomic analysis of the iMSMS cohort demonstrated global enrichment of core urease genes in PMS. Previous work indicates that bacterial urease alters gut luminal pH and induces T cell-dependent intestinal inflammation (Ni et al., 2017).

Conclusion: We identify a urease-expressing clade of *H. hathewayi* that is robustly associated with progressive MS across international cohorts. These findings reveal a microbiome-driven metabolic pathway potentially contributing to PMS pathogenesis and highlight bacterial urease as a promising therapeutic target.

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Designing Stable Food Ingredients Containing a Wild-Type Presumptive Probiotic Strain

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Despite the growing interest in functional foods, maintaining high levels of viable beneficial cells during digestion and storage remains a major bottleneck for the food industry. Meanwhile, survival during gastrointestinal transit is considered a prerequisite for probiotic efficacy. Cell immobilization has emerged as a promising strategy for maintaining cell viability, whereas freeze-dried cultures are generally preferred by the food industry. In this vein, our objective was to develop stable freeze-dried food ingredients bearing high cell loads of the wild-type presumptive probiotic strain *Lactiplantibacillus plantarum* V2. Cell immobilization on oat flakes, banana powder, and hazelnut was applied, and cell viability was monitored during an in vitro digestion model, as well as during storage at room and refrigerated temperatures for up to 6 months. During *in vitro* digestion, all immobilized cells exhibited higher survival rates (> 80.15%) compared to free cells (< 56.35%). After 6 months of storage, freeze-dried immobilized cells exhibited cell loads $\geqslant$ 7.1 logcfu/g at room temperature and $\geqslant$ 8.2 logcfu/g under refrigerated conditions, while the cell viability of freeze-dried free cells was < 7 logcfu/g at both temperatures. Furthermore, freeze-drying led to higher cell levels compared to wet cultures during storage at both temperatures, as expected. These findings highlighted the effectiveness of cell immobilization, demonstrating their potential to preserve probiotic viability and support the development of stable functional food ingredients that could be incorporated into food systems.

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Acquisition of a Gene Cluster in *Clostridioides difficile* PCR Ribotype 023 Strains Confers Xylitol Utilization Ability

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Hundreds of ribotypes of the gastrointestinal pathogen *Clostridioides difficile* have emerged over the last three decades, yet the factors driving their emergence are poorly understood. Recently, there has been an increase in infections caused by PCR ribotype 023 (RT023) strains in Europe. We profiled the growth of 7 RT023 strains on 190 unique carbon sources and found they were able to grow on xylitol, a sugar alcohol commonly used as a food additive for humans and animals. Other ribotypes of *C. difficile* tested (n=19) displayed little to no growth in 0.5% xylitol and were growth inhibited in higher concentrations of xylitol. Genome sequencing identified that RT023 strains acquired a gene with high similarity to xylitol dehydrogenase (xdh) in a mobile genetic element (MGE) that is absent from other *C. difficile* ribotypes. We created a clean deletion of xdh in the RT023 strain PRB1128 and observed poor growth on xylitol, indicating that *xdh* is required for xylitol utilization. Complementation of *xdh* using an inducible plasmid restored growth on xylitol. We performed *C. difficile* strain competition assays in minibioreactor arrays (MBRAs) and observed PRB1128 outcompeted a non-xylitol utilizing strain (RT027, CD2015) in the presence of xylitol. In addition, this strain stably colonized a fecal community in MBRAs but colonization did not require xylitol. These data suggest that the *xdh* contained within RT023s provides a fitness benefit for growth on xylitol. Interestingly, the chromosomal locus where the MGE inserted appears to be a hotspot for genetic insertions across clades of *C. difficile*. Together, this work improves our understanding of the molecular basis for niche adaptation of *C. difficile*.

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