# PROGRAM & ABSTRACT BOOK

WELCOME

JULY 9-12, 2018

# ANAEROBE 2018

*Las Vegas, Nevada*

## The 14th Biennial Congress of the Anaerobe Society of the Americas

***Tropicana Hotel***

***Las Vegas, Nevada***

# Partnership opportunities with Sanofi Pasteur

**Sanofi Pasteur is interested in potential partnering opportunities in the field of active and passive human immunization, as well as technologies supporting product development and industrial performance, including the following areas:**

**Vaccines, monoclonal antibodies and supporting technologies for prevention and treatment of infectious diseases**

- Novel antigens and methods for antigen discovery and characterization
- Vaccine vectors suitable for nasal or oral use
- New ways to administer vaccines
- Carrier proteins and protein-polysaccharide conjugation methods or alternative technologies

**Agents to enhance vaccine immune responses**

- Adjuvants and immunomodulators
- Vaccine vectors and delivery systems intended to enhance or modify immune responses
- Biological and immunological studies to further characterize adjuvants and immunomodulators

**Characterization and assay of immune responses and disease markers**

- Animal models of human diseases
- Biological markers for evaluating the efficacy of prophylactic or therapeutic interventions
- *In vitro* models of human tissues, including the immune system
- Epidemiological studies relevant to the use of vaccines and immunotherapeutics

**Tools for improving vaccine and monoclonal antibody research, development and production**

- Development and application of new technologies in the areas of genomics and proteomics
- Prokaryotic or eukaryotic cell lines for antigen production
- Fermentor and bioreactor technology
- Disposable systems
- Online testing
- Downstream processing, purification and aseptic filling processes
- Process automation
- Preservatives and stabilizers
- Bioinformatics techniques for modeling, data handling and analysis
- Anti-counterfeiting technology

## CONTACT DETAILS

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SANOFI PASTEUR

# PROGRAM & ABSTRACT BOOK


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***The 14th Biennial Congress of the Anaerobe Society of the Americas***
***Tropicana Hotel • Las Vegas, Nevada USA***


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Welcome to ***ANAEROBE 2018***, the 14th biennial Congress of the Anaerobe Society of the Americas (ASA)! This forum brings together clinicians and scientists from around the world to engage in presentations, dialogue, and interaction related to the clinical and microbiological aspects of anaerobic bacteriology. The Congress will explore the role of anaerobes in both health and disease, while addressing traditional and emerging technologies for identification and diagnosis. ***ANAEROBE 2018*** again illustrates the international interest in the field of anaerobic bacteriology: 171 abstracts were submitted for presentation, representing the work of over 600 scientists from nearly 30 countries.

The *Keynote Address* will be given by MacArthur Foundation Fellow **Sarkis Mazmanian, Ph.D.** of the California Institute of Technology. Dr. Mazmanian will discuss the molecular mechanisms of the gut microbiome associated with inflammatory bowel disease.

The *Lifetime Achievement Award* will be presented to **Ellen Jo Baron, Ph.D.** of Stanford University and Cepheid. Dr. Baron is being recognized for her outstanding career contributions to the fields on anaerobic bacteriology and infectious diseases.

We would like to thank the members of the Organizing Committee and the Session Chairs for their assistance in formulating what promises to be another exciting program. We also would like to thank those from industry—both patrons and exhibitors listed on page v—for the financial support that makes this Congress possible, as well as grants from the Burroughs Welcome Fund, Merck, Sanofi-Pasteur, Pfizer, and the European Society of Clinical Microbiology and Infectious Diseases.

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

Very special thanks goes to Dr. Ronald and Pamela Goldman, who again have done an exemplary job in bringing this meeting together.

Finally, please take the opportunity to explore and experience the glitz, glamour, and gaudiness of America's entertainment capital!

Our hope is that ***ANAEROBE 2018*** serves to foster stimulating discussions, as well as cultivate personal relationships that continue to invigorate the entire field beyond the timeframe of this Congress.

*Jeanne Marrazzo, M.D.*
ASA President

## About the Anaerobe Society

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

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

## Anaerobe Society Congresses

This is the 14th biennial Anaerobe Society Congress.

Past Anaerobe Society sponsored programs were:

**ANAEROBE 2016—Nashville, TN USA**

**ANAEROBE 2014—Chicago, IL USA**

**ANAEROBE 2012—San Francisco, CA USA**

**ANAEROBE 2010—Philadelphia, PA USA**

**ANAEROBE 2008—Long Beach, CA USA**

**ANAEROBE 2006—Boise, ID USA**

**ANAEROBE 2004—Annapolis, MD USA**

**ANAEROBE OLYMPIAD 2002—Park City, UT USA**

**2001: AN ANAEROBE ODYSSEY—Los Angeles, CA USA**

**ANAEROBE 2000—Manchester, England**

**ANAEROBE 1998—Buenas Aires, Argentina**

**ANAEROBE 1996—Chicago, IL USA**

**ANAEROBE 1994—Los Angeles, CA USA**

**ANAEROBE 1992—Los Angeles, CA USA**

Anaerobe Society of the Americas gratefully acknowledges the following organizations for their generous support of this congress.

Support for this activity was received in the form of educational grants from:

- Burroughs Wellcome Fund
- European Society of Clinical Microbiology and Infectious Diseases

Support for this activity from commercial interests include:

### Platinum Patrons

- Merck
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- Acurx Pharmaceuticals
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- Summit Therapeutics

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- Anaerobe Systems
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- Advanced Instruments
- Cayman Chemicals
- Clostridium Difficile Foundation
- Gut Check Foundation
- Key Scientific Products
- Microbiology International
- Plas-Labs
- Seres Therapeutics
- Shel Lab

Photo: John D. & Catherine T. MacArthur Foundation

## Sarkis Mazmanian, Ph.D.

Dr. Mazmanian is the Luis B. and Nelly Soux Professor of Microbiology in the Division of Biology & Biological Engineering at the California Institute of Technology and an Investigator of the Heritage Medical Research Institute.

He began his college career at the University of California, Los Angeles (UCLA) with the intent of becoming an English major, but after exposure to the microscopic world in the biology lab, he "never looked back."

He earned his Ph.D. at UCLA in 2002—under the tutelage of Dr. Olaf Schneewind—focusing on the virulence factors related to *Staphylococcus aureus.* With a *Helen Hay Whitney Postdoctoral Fellowship,* his interests shifted to gut bacteria, joining the lab of Dr. Dennis Kasper at Harvard University to study *Bacteroides fragilis.* In 2006, he established his own lab at Cal Tech, where he has taken a broader look at the gut microbiome and potential link to Inflammatory Bowel Disease, Multiple Sclerosis, Parkinson's disease, and Autism.

*Immunologic and neurologic imbalances underlie many diseases. The human body represents a scaffold upon which multitudes of commensal species build residence, creating a diverse ecosystem with members of five of the six kingdoms of life. Mechanisms that mediate the interdependent and complex interactions between the microbiome and animals, as well as their influences on human health, represent an exciting frontier of science and medicine. Our laboratory aims to discover how gut bacteria influence the development and function of the immune and nervous systems, with the goal of understanding mechanisms by which the microbiome contributes to the critical balance between health and disease.*

For his work, Dr. Mazmanian has received numerous honors, including the *Burroughs Wellcome Fund's Investigators in the Pathogenesis of Infectious Disease*, (2011), a MacArthur Foundation "genius" grant (2013), and named by *Discover Magazine* as one of the "Best Brains in Science under 40."

He is a founder of two biotech companies and has or currently serves on the Scientific Advisory Board of over a dozen companies, academic centers, and not-for-profit foundations.

Besides his research at Cal Tech, Dr. Mazmanian travels annually to Armenia to teach microbiology at the Institute of Molecular Biology in the city of Yerevan. Though the building is dilapidated, "the will of the students and their desire to learn is incredible." He feels an obligation to support the teaching of life science in his country of origin. "I feel very grateful to be in a position to help people who are not as educationally fortunate as students in this country."

## Ellen Jo Baron, Ph.D.

Dr. Baron holds a Ph.D. in Medical Microbiology from the University of Wisconsin, Madison (1981), and completed a post-doctoral fellowship in Clinical Microbiology and Laboratory Medicine at the University of California, Los Angeles (UCLA) and the Wadsworth Veterans Affairs Medical Center in Los Angeles, CA (1983). She was Chief Microbiologist at North Shore University Hospital in Manhasset, NY, Director of the Wadsworth Anaerobe Clinical Laboratory in Los Angeles, CA, Clinical Associate Professor at UCLA and University of Southern California, and consultant to various Southern California hospitals.

In 1997, she joined the clinical faculty of Stanford University in Microbiology and Pathology and served as Director of the Clinical Microbiology & Virology Laboratories and Associate Chair of Pathology for Faculty Development and Diversity in the School of Medicine. In 2009, she became *Professor Emerita*, when she went into industry, becoming Cepheid's Director of Medical Affairs, (2008-2013), Executive Director of Technical Support, Cepheid (2013-2015) and back to Medical Affairs (2015-2018).

Among her achievements are the discovery and characterization (with Julie Downes) of the anaerobic bacterium *Bilophila wadsworthia* and co-developer of programs for antimicrobial resistance detection and basic microbiology for implementation in the developing world, now being developed through the World Health Organization. She has written or edited over 30 books and chapters, is widely published in peer-reviewed journals in the area of diagnostic microbiology and infectious diseases (with over 100 publications), and has lectured at conferences and schools around the world.

Dr. Baron has served on the editorial boards of several key clinical microbiology journals and was a volume editor (Bacteriology) for the ASM's *Manual of Clinical Microbiology* for 4 editions.

She was the Microbiology representative on the Center for Medicaid and Medicare Services Clinical Laboratory Improvement Advisory Committee, a member of the Council of Clinical Advisors for the NIH Clinical Laboratories, a representative on the Clinical Laboratory Standards Institute's Microbiology Area Resource Committee, Chairperson of the Clinical Microbiology Division of the American Society for Microbiology, and Chair of the American Board of Medical Microbiology.

Her honors include the American Society for Microbiology's *Alice C. Evans Award* (1997), *BioMerieux Sonnenwirth Award for Leadership* (2000), and Founders's Distinguished Service Award; the Stanford University's *Kenneth L. Vosti, M.D. Teaching Award* (2003), *Stanford Medicine Lifetime Achievement Award* (2016), Fellow status in both the American Academy of Microbiology and Infectious Diseases Society of America, and Diplomate of the American Board of Medical Microbiology.

***ANAEROBE 2018***—the 14th biennial Congress of the Anaerobe Society of the Americas—provides the forum for vigorous discussions of both the clinical and microbiological aspects of anaerobic infections, their diagnosis, and their therapy among medical practitioners, researchers, and laboratory scientists.

## Physician Accreditation

No Physician Continuing Medical Education Units will be issued for the Congress. Attendees may request Certificates of Attendance, free of charge (see below).

## Clinical Laboratory Scientist Accreditation

Microbiology Educational Services is accredited by the California Department of Health Services to provide continuing education for clinical laboratory scientists.

Microbiology Educational Services designates this educational activity for a maximum of 21.0 continuing education contact hours upon completion of the program and 7.0 continuing education contact hours upon completion of each workshop. Clinical laboratory scientists should claim only those hours of credit that they actually spent in the educational activity.

## Certificates of Attendance

Certificates of Attendance may be requested on the Evaluation Form. Completed Evaluation Forms, for sessions attended, must be returned to the Registraton Table before departing the Congress. Certificates will be emailed to attendees.

## Curricular Goals & Objectives

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

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

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

## Disclosures

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

## Evaluation Forms

Please complete the Evaluation Form in your Delegate Packet and return it to the Registration Table at the completion of the Congress.

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

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

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

The disclosure information received from each individual is presented on the following pages. All disclosure information has been reviewed for conflict of interest by the ASA Program Committee. Conflicts identified and resolved are noted below. If no notation is made, a conflict of interest was not in existence.

## Participant Disclosure

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

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The following presenters have information to disclose as follows:

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| Paul G. Auwaerter, M.D. | Johnson & Johnson (O) |
| Ken Blount, Ph.D. | Rebiotix (E)<br>*Investigational Drug* |
| Nathan S. Bryan, Ph.D. | HumanN (C, O) Saje Pharma (C, O) |
| Karen Carroll, M.D. ` | Accelerate (G), Curetis (G), GenePOC (G), GenMark (G) |
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| Sathursha Gunaratnam | BioK+ International (G) |
| George Hajishengallis, D.D.S., Ph.D. | None<br>*Complement inhibitor (Cp40) used to inhibit periodontitis in animal model* |
| Stuart Johnson, M.D. | BioK+ (C), Cutis (C), Synthetic Biologics (C), Summit (C) |
| Sahil Khanna, M.B.B.S., M.S. | Facile Therapeutics (C), Merck (C), ProBio Tech (C), Premier (C), Rebiotix (C, G), Shire (C) |
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A=Advisory Board, C=Consultant, E-Employment, G=Grant, O=Ownership/Stock, P=Patent, R-Royalty, S=Speaker

## Monday, July 9

0800

**WORKSHOPS & CONGRESS REGISTRATION OPENS**

0900-1700

**ANAEROBIC IDENTIFICATION & SUSCEPTABLITY WORKSHOP**

*Diane M. Citron*

*Mike Cox*

0900-1700

**EXAMINING ANAEROBES IN THE MICROBIOME: METAGENOMIC AND CULTURE APPROACHES**

*Laura M. Cox, Ph.D.*

*Casey M. Theriot, Ph.D*

*Anna M. Seekatz, Ph.D.*

1800

**BREWS, WINE & WINGS AT ROBERT IRVINE'S PUBLIC HOUSE**

**Tropicana Hotel**

## Tuesday, July 10

**0700-0820 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS**

**0820**

**WELCOME REMARKS**

*Jeanne Marrazzo, M.D., ASA President*

**0830-0945**

**SESSION I: ONE HEALTH: SYNERGY BETWEEN HUMAN & ANIMAL ECOLOGIC SYSTEMS**

*Convener: Francisco A. Uzal, Ph.D.*

SI-1 Pathogenesis of *Clostridium perfringens* Type C Infections in Humans and Animals
*Jihong Li, Ph.D.*

SI-2 Pathobiology of Clostridial Hepatitis in Animals
*Mauricio Navarro, D.V.M., M.Sc.*

SI-3 *Brachyspira* Species Infections in Animals and Humans
*Eric R. Burrough, D.V.M., Ph.D.*

**0945-1000 BREAK / INDUSTRY EXHIBITS**

**1000-1100**

**SESSION II: KEYNOTE PRESENTATION**

*Convener: Jeanne Marrazzo, M.D.*

SII-1 Microbiota and the Brain-Gut Axis
*Sarkis K. Mazmanian, Ph.D.*

**1100-1200**

**SESSION III: ANAEROBIC CLINICAL ISSUES IN HUMAN DISEASE**

*Convener: Ellie J.C. Goldstein, M.D.*

SIII-1 The Role of Anaerobes in Pneumonia: A Breath of Fresh Air?
*Stephen G. Jenkins, Ph.D.*

SIII-2 *Cutibacterium* (ex. *Propionibacterium) acnes* and Shoulder Surgery
*Paul G. Auwaerter, M.D.*

SIII-3 Characterization of *Cutibacterium (ex. Propionibacterium) acnes* Populations in Patients with Acne *versus* Healthy Skin: Interest of a Local Adjunctive Therapy Based on Myrtacine
*Christine G. Roques, Ph.D.*

**1200-1320 LUNCH / INDUSTRY EXHIBITS STUDENT COMPETITION PRESENTATIONS**

**1320-1415**

**SESSION IV: SYSTEMS BIOLOGY TO STUDY THE MICROBIOME & INFECTIOUS DISEASES**

*Convener: Casey M. Theriot, Ph.D.*

SIV-1 Extracting Actionable Knowledge from the EHR: Machine Learning Tools for Predicting Risk of CDI
*Jenna Wiens, Ph.D.*

SIV-2 Mathematical Modeling of *C. difficile* Infection and Antimicrobial Resistance
*Cristina Lanzas, D.V.M., Ph.D.*

## Tuesday, July 10

**SESSION V: NEW TOPICS IN ANAEROBIC SCIENCE** 1415-1510

*Convener: Yiping Han, Ph.D.*

SV-1 The Development of *Clostridium difficile* Bacteriophages for Therapeutic Purposes
*Martha R.J. Clokie, Ph.D.*

SV-2 Engineering Regulatory Systems to Modulate Gene Expression of *Bacteroides* in the Gut
*Bentley Lim, Ph.D.*

**1510-1530 BREAK / INDUSTRY EXHIBITS**

**SESSION VI: GENITAL TRACT ANAEROBES, HIV/STI ACQUISITION & REPRODUCTIVE HEALTH** 1530-1645

*Convener: David Fredricks, M.D.*

SVI-1 Microbiome Studies to Define the Role of Vaginal Anaerobes in Increasing Susceptibility to HIV
*Sujatha Srinivasan, Ph.D.*

SVI-2 The Virtue of Simplicity: The Vaginal Microbiome, Genital Inflammation and HIV Risk
*Douglas S. Kwon, M.D., Ph.D.*

SVI-3 Anaerobes and Immunity in the Female Genital Tract
*Caroline M. Mitchell, M.D.*

**SESSION VII: ORAL PRESENTATIONS I—INTERNATIONAL PERSPECTIVES** 1645-1800

*Convener: Daniel Paredes-Sabja, Ph.D.*

SVII-1 Ongoing Research on Anaerobes in Europe
*Elisabeth Nagy, Ph.D.*

SVII-2 UK *Bacteroides* Species Surveillance Survey: Change in Antimicrobial Resistance Over 16 Years
*Harriett C. Hughes, Ph.D.*

SVII-3 *Clostridioides difficile:* What Do We Know about this Pathogen in Brazil?
*Eliane O. Ferreira, Ph.D.*

SVII-4 New Insights into *Clostridium difficile* (CD) Infection in Latin America: Novel Description of Toxigenic Profiles of Diarrhea-Associated CD in Bogotá, Colombia
*Marina Muñoz, Ph.D.*

**WINE & CHEESE RECEPTION** 1800-1900
**Exhibit Hall**

## Wednesday, July 11

**0700-0745 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS**

0745-0845

**SESSION VIII: ORAL PRESENTATIONS II—SEQUENCING & PROBIOTICS**

*Convener: Ernesto Abel-Santos, Ph.D.*

SVIII-1 Challenges of Next-Generation-Sequencing Targeting Anaerobes
*Georg Conrads, Ph.D.*

SVIII-2 Phylogenomic Analysis of *Fusobacterium necrophorum* Based on Whole Genome Sequencing and Its Association with Disease and Host
*Anders A. Jensen, Ph.D.*

SVIII-3 *Lactobacillus* Cocktails Modulate Monocyte Immune Responses to Pathogenic Vaginal Anaerobes
*Hidemi Yamamoto*

SVIII-4 *Clostridium difficile* Virulence Factors Impaired by BIO-K+ Probiotics
*Sathursha Gunaratnam*

**0845-0900 BREAK / INDUSTRY EXHIBITS**

0900-1020

**SESSION IX: THE ORAL MICROBIOME & HUMAN HEALTH**

*Convener: Purnima Kumar, D.D.S.*

SIX-1 *P. gingivalis* as an Orchestrator of Dysbiosis in Periodontitis
*George Hajishengallis, D.D.S., Ph.D.*

SIX-2 Targeting of the Iron-Dependent Metabolism of Oral Anaerobic Bacteria
*Janina Lewis, Ph.D.*

SIX-3 SyngenicDNA: Making Every Bacterial Species Genetically Tractable
*Christopher D. Johnston, Ph.D.*

SIX-4 Nitrosative Stress Sensing in *Porphyromonas gingivalis*: Structure and Function of the Heme Binding Transcriptional Regulator HCPR
*Benjamin Ross Belvin*

**1020-1045 BREAK / INDUSTRY EXHIBITS**

1045-1200

**SESSION X: *FUSOBACTERIUM* AND PATHOGENESIS**

*Convener: Sarah Kuehne, Ph.D.*

SX-1 New Insights into *Fusobacterium*-Associated Colorectal Cancers
*Susan Bullman, Ph.D.*

SX-2 *Fusobacterium* in the Oral Cavity and Its Signaling Potential
*Sarah A. Kuehne, Ph.D.*

SX-3 Oral Bacteria and CRC: What is Their Role?
*Julia L. Drewes, Ph.D.*

SX-4 *Fusobacterium nucleatum* and Adverse Pregnancy Outcomes: A Review of Epidemiological and Mechanistic Evidence
*Yiping W. Han, Ph.D.*

**1200-1300 LUNCH / INDUSTRY EXHIBITS**

**1300-1400 POSTER SESSION I / INDUSTRY EXHIBITS**

## Wednesday, July 11

1400-1520

**SESSION XI: DOES THE HUMAN MICROBIOME IMPACT TREATMENT OF NON-INFECTIOUS DISEASE?**

*Convener: Cynthia Sears, M.D.*

SXI-1 The Gut Microbiome Affects the Uptake and Metabolism of Drugs
*Jordan E. Bisanz, Ph.D.*

SXI-2 The Contribution of Oral Anaerobes to Hypertension: Is There NO Link?
*Nathan S. Bryan, Ph.D.*

SXI-3 Anaerobes and the Brain: The Role of Commensal Microbiota in Neurologic Disease
*Laura M. Cox, Ph.D.*

**1520-1540 BREAK / INDUSTRY EXHIBITS**

1540-1630

**SESSION XII: TAXONOMY CHANGES AND THE CLINICAL IMPLICATIONS**

*Convener: Diane Citron*

SXII-1 Winds of Change: Taxonomic Restructuring of the Clinically Important Clostridia
*Paul A. Lawson, Ph.D.*

SXII-2 Clinical Implications of Anaerobic Taxonomy Changes
*Karen C. Carroll, M.D.*

1630-1800

**SESSION XIII: ORAL PRESENTATIONS III: *CLOSTRIDIOIDES (CLOSTRIDIUM) DIFFICIL*—LABORATORY RESEARCH**

*Convener: Stuart Johnson, M.D.*

SXIII-1 Extracellular Vesicles Produced by *Clostridioides difficile*
*Leandro A. Lobo, Ph.D.*

SXIII-2 Characterization of Germination Inhibitors against *Clostridium difficile* R20291
*Christopher Yip*

SXIII-3 Persistence of *Clostridium difficile* Spores in the Intestinal Epithelium and Its Role in Recurrent Disease
*Daniel Paredes-Sabja, Ph.D.*

SXIII-4 Microbiota of Mucosal Associated Inveriant T Cell Deficient Mice Confer Resistance against *Clostridium difficile* Infection
*Ashley D. Smith, Ph.D.*

SXIII-5 IL-22 Prevents *Clostridium difficile* Infection via Modulation of Microbial Metabolic Activities in the Gut
*Nobuhiko Kamada, Ph.D.*

SXIII-6 Obesity-Associated Gut Microbiota Enhances *Clostridium difficile* Infection in Mice
*Shinsmon Jose, Ph.D.*

**1830 CONGRESS RECEPTION / HAVANA ROOM**

**1915 CONGRESS BANQUET & AWARDS / HAVANA ROOM**

## Thursday, July 12

**0700-0800 REGISTRATION / BREAKFAST / INDUSTRY EXHIBITS**

0800-0900

**SESSION XIV: ORAL PRESENTATIONS IV: *CLOSTRIDIOIDES (CLOSTRIDIUM) DIFFICILE*—CLINICAL TRIALS I**

*Convener: Glenn S. Tillotson, Ph.D.*

SIV-1 Insights from Fidaxomicin, Bezlotoxumab and Surotomycin Clinical Trials: Looking Beyond the Primary Analyses
*Mary Beth Dorr, Ph.D.*

SIV-2 SYN-004 (Ribaxamase) Prevents *Clostridium difficile* Infection and Antimicrobial Resistance
*John F. Kokai-Kun, Ph.D.*

SIV-3 The Microbiota-based Drug RBX2660 is Efficacious and Safe in Patients with Recurrent *Clostridium difficile* Infections: Results from 2 Controlled Clinical Trials
*Dale N. Gerding, M.D.*

SIV-4 Misoprostol Protects Mice against *Clostridium difficile* Infection and Accelerates Recovery of Gut Microbial Diversity After Antibiotics
*David M. Aronoff, M.D.*

**0900-0910 BREAK / INDUSTRY EXHIBITS**

0910-1020

**SESSION XV: THE "NON-*DIFFICILE*" *CLOSTRIDIA*: CURRENT CLINICAL & THERAPEUTIC LANDSCAPE**

*Convener: Dennis Stevens, M.D.*

SXV-1 Enterotoxin-Producing *Clostridium perfringens* Type F: Molecular Mechanisms and Clinical Syndromes
*Bruce A. McClane, Ph.D.*

SXV-2 Botulinum Neurotoxin-Encoding Plasmids Can Be Congugatively Transferred to Diverse *Clostridial* Strain
*Eric A. Johnson, Sc.D.*

SXV-3 The Identification and Characterization of a Novel Extracellular Metalloproteinase Produced by *Clostridium sordellii*
*Michael J. Aldape, Ph.D.*

SXV-4 *Clostridia*, Preterm Neonates and Necrotizing Enterocolitis: New Data
*Julio Aires, Ph.D.*

**1020-1030 BREAK / INDUSTRY EXHIBITS**

1030-1200

**SESSION XVI: AN UPDATE ON *CLOSTRIDIUM DIFFICILE* PATHOGENESIS**

*Convener: David Aronoff, M.D.*

SXVI-1 An Update on the Mechanisms of Action of the Large *C. difficile* Toxins
*D. Borden Lacy, Ph.D.*

SXVI-2 Host Immune Defense to *C. difficile* Infection
*William A. Petri, Jr., M.D., Ph.D.*

SXVI-3 Structure-function Analyses of the Putative *Clostridium difficile* Bile Salt Germinant Receptor, CspC
*Amee Shen, Ph.D.*

SXVI-4 Alterations in the Gut Metabolome and *Clostridium difficile* Transcriptome in a Mouse Model of Infection
*Josh R. Fletcher, Ph.D.*

## Thursday, July 12

**1200-1300 LUNCH / INDUSTRY EXHIBITS**

**1300-1400 POSTER SESSION II / INDUSTRY EXHIBITS**

**SESSION XVII: *CLOSTRIDIUM DIFFICILE*—EVOLVING MANAGEMENT**

*Convener: Dale N. Gerding, M.D.*

SXVII-1 New IDSA/SHEA Guidelines for CDI Diagnosis and Treatment
*Stuart Johnson, M.D.*

SXVII-2 Fecal Microbiota Transplant and Derivatives for CDI
*Sahil Khanna, M.B.B.S., M.S.*

SXVII-3 Update on Newer Management of *Clostridium difficile* Infection (CDI)
*Dale N. Gerding, M.D.*

1400-1515

**1515-1530 BREAK**

**SESSION XVIII: ORAL PRESENTATION V: *CLOSTRIDIUM DIFFICILE*—CLINICAL TRIALS II**

*Convener: Vincent Young, M.D., Ph.D.*

SXVIII-1 Oral Beta-Lactamase Therapy Protects the Gut Microbiome from IV and Oral Antibiotics and Mitigates Propagation of Antibiotic Resistance
*Sheila Connelly, Ph.D.*

SXVIII-2 Developing Microbiome Rehabilitation Biomarkers for *Clostridium difficile* Infections: Continued Evaluation of a Prototype Microbiome Health Index
*Ken Blount, Ph.D.*

SXVIII-3 Gastrointestinal Tract Microbiome Dynamics Following Treatment with SER-109; An Investigational Oral Microbiome Therapeutic to Reduce the Recurrence of *Clostridium difficile* Infection (CDI)
*Christopher B. Ford, Ph.D.*

SXVIII-4 Ridinilazole (RDZ) for *Clostridium difficile* Infection (CDI)
*Sumita Chowdhury, M.D.*

SXVIII-5 How About Progress Towards the Development of a Prophylactic *Clostridium difficile* Vaccine
*Annaliesa S. Anderson, Ph.D.*

1530-1645

**CLOSING COMMENTS**

**CONGRESS CONCLUDES**

1645-1700


WELCOME
JULY 9-12, 2018
ANAEROBE 2018
Las Vegas, Nevada

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

Abstracts are identified by:

Session Number (in Roman numerals)

Type of Paper
- S—Faculty/Oral Presentation
- PI—Poster Presentation/Session I
- PII—Poster Presentation/Session II
- SP—Student Presentation

*Indicates Presenting Author

Refer to the Program Section of this book (pages xv-xxi) for presentation times.

***0830*** ***SESSION I:*** **ONE HEALTH: SYNERGY BETWEEN HUMAN & ANIMAL ECOLOGIC SYSTEMS**

*SI-1*

## PATHOGENESIS OF *CLOSTRIDIUM PERFRINGENS* TYPE C INFECTIONS IN HUMANS AND ANIMALS

Li, J.;*[1] McClane, B.A.;[1] Uzal, F.A.[2]
[1]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA USA
[2]California Animal Health and Food Safety Laboratory, University of California Davis, San Bernadino, CA USA

*Clostridium perfringens* type C stains are human and animal enteropathogens, causing necrotic enteritis (i.e. enteritis necroticans –EN) and enterotoxemia. In the 1970s-80s EN was the most common cause of childhood death in the Papua New Guinea highlands, where it is now re-emerging. EN outbreaks also develop sporadically in malnourished people in other regions of the world. In the USA, individual EN cases occur in people with diabetes, an increasingly prevalent disease. EN is usually a foodborne disease caused by strains producing highly-resistant spores. Type C strains also cause enteritis and enterotoxaemia in livestock, including sheep, goats, horses and pigs. When causing enteritis or enterotoxemia, type C strains must produce beta toxin (CPB), but they can also make CPE or TpeL. Molecular Koch's postulates analyses demonstrated that CPB is essential for the type C strain CN3685 pathogenies in rabbits and goats. CPB can affect the epithelium of the jejunum, ileum and to a lesser extent, the duodenum and colon, where it causes fluid accumulation and necrosis of the intestine with variable hemorrhage. During enterotoxemia, CPB is absorbed into the circulation and then damages internal organs, such as the brain. Other molecular Koch's postulate analyses provided the evidence that both CPB and CPE can act synergistically during EN pathogenesis. Since CPB is sensitive to trypsin, trypsin is a key innate defense against disease mediated by CPB. There are two natural variant CPB toxins with four conserved amino acid changes; one of them demonstrates a significant difference in trypsin sensitivity.

Most type C EN strains also produce up to three sialidases, NanJ, NanI and NanH. A recent study demonstrated that NanI sialidase can increase the binding and cytotoxic activity of CPB and CPE. NanI sialidase also can be activated by trypsin, chymotrypsin or intestinal fluid, and furthermore, this activation of NanI further increased CPE and CPB binding and cytotoxic activity.

Both VirR/S two-component system and Agr-like quorum sensing system are essential for CPB expression and mediate enteritis and/or enterotoxemia diseases. Inhibitors of signal peptide signaling can attenuate virulence and are promising therapeutic candidates for type C diseases.

## PATHOBIOLOGY OF CLOSTRIDIAL HEPATITIS IN ANIMALS

Navarro, M.A.;* Uzal, F.A.
California Animal Health and Food Safety Laboratory System, San Bernardino Branch, University of California Davis, San Bernadino, CA USA

While many clostridial diseases affect humans and animals, clostridial hepatitis seem to be restricted to veterinary medicine. *Clostridium novyi* type B is a gram-positive anaerobe that causes infectious necrotic hepatitis (INH) in sheep, and less frequently in cattle and horses. Spores of *C. novyi* can be present in soil, and after being ingested they can reach the liver *via* portal circulation and persist in phagocytic cells. Following liver damage, frequently caused by migrating parasites, local anaerobic conditions allow the germination of the spores and the production of toxins. *C. novyi* type B alpha toxin causes necrotizing hepatitis in the liver and extensive infiltration of fluids, congestion and hemorrhages in distant organs. *Clostridium haemolyticum* causes bacillary hemoglobinuria (BH) in cattle, sheep, and sometimes horses. This gram-positive microorganism is one of the strictest anaerobic pathogenic clostridia. The pathogenesis of BH shares many similarities with INH, in which an initial focus of hepatic necrosis and anaerobiosis is needed for germination of spores and production of toxins. Of these, beta toxin is considered the main virulence factor responsible for not only hepatic necrosis, but also for the intense hemolysis observed in affected animals, resulting in anemia, hemoglobinuria and icterus. *Clostridium piliforme*, the causal agent of Tyzzer's disease, is the only gram-negative and obligate intracellular member of the clostridial family. The disease is more frequent in foals, lagomorphs and laboratory animals. Though little is known about the pathogenesis of *C. piliforme* infection, it is believed that the mode of transmission is fecal-oral, by ingestion of spores from an environment contaminated by feces. In immunocompromised animals, *C. piliforme* proliferates in the intestinal mucosa, resulting in necrosis and dissemination to the liver and other organs. Virulence factors for this microorganism have not been identified. Due to the peracute or acute nature of clostridial hepatitis in animals, treatment is rarely effective. The incidence and severity of these conditions should be controlled by the use of bacterin and toxoid vaccines available for INH and BH, and by preventive environmental measures in all cases.

*SI-3*

## *BRACHYSPIRA* SPECIES INFECTIONS IN ANIMALS AND HUMANS

Burrough, E.R.*
Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA USA

Currently there are nine officially named species in the genus *Brachyspira.* These anaerobic spirochetes colonize the large intestines of multiple animal species and humans resulting in a range of outcomes from inapparent infections to severe clinical disease depending upon the bacterial species and a variety of host factors. This presentation will discuss the common host range of recognized species, typical disease manifestations, and recent trends in diagnostic detection and differentiation methods.

*Brachyspira pilosicoli* has the broadest host range and has zoonotic potential. Intestinal spirochetosis has been described in pigs, poultry, and humans in association with *B. pilosicoli* infection and isolates of human origin have been experimentally inoculated into pigs, chickens, and mice. Pigs are a major host for *Brachyspira* spp. and can be colonized by at least seven of the officially named species. The most significant disease in pigs, swine dysentery, is a severe mucohemorrhagic colitis caused by strongly beta-hemolytic isolates of *Brachyspira hyodysenteriae*, *Brachyspira hampsonii*, or *Brachyspira suanatina.* In chickens, infections with *Brachyspira intermedia*, *Brachyspira alvinipulli*, and *B. pilosicoli* have been associated with diarrhea and reduced egg production.

A major challenge in the diagnosis of *Brachyspira*-associated disease has historically been lack of detection by routine cultural methods due to the specialized growth requirements of these spirochetes. Selective anaerobic culture is required for isolation from clinical specimens and the organisms are slow growing, often taking three to five days to form a thin film of surface growth in association with varying degrees of beta-hemolysis on blood agar. Accordingly, *Brachyspira* spp. may go undetected in poly-microbial infections or simply not be investigated. Diagnosis frequently follows observation of typical spirochetes in histopathologic sections and many polymerase chain reaction assays are available for detection in clinical specimens. While PCR assays have made detection faster and more broadly available, culture remains valuable in determining the hemolytic phenotype of the spirochetes and for confirming viability.

***1000*** ***SESSION II:*** **KEYNOTE ADDRESS**

*SII-1*

## MICROBIOTA AND THE BRAIN-GUT AXIS

Mazmanian, S.K.*
California Institute of Technology, Pasadena, CA USA

Imbalances in the immune system cause autoimmune, allergic, and inflammatory disorders that impact millions worldwide. Gut microbiota control the development and function of the immune system, and play a critical role in inflammatory bowel disease (IBD), a family of idiopathic intestinal disorders with increasing prevalence and limited treatment options. Concordance rates of 30-40% between monozygotic twins implicate gene-environment interactions contribute to IBD, albeit in ways that are poorly understood. Advances in DNA sequencing technologies have empowered unprecedented insights into the human genome and the gut microbiome in IBD enabling detailed genomic characterization of patients and chronicling alterations in the composition and gene content of the gut microbiome (dysbiosis). However, molecular mechanisms unifying beneficial gene-microbiota interactions remain largely undescribed. The human commensal *Bacteroides fragilis* produces immunomodulatory molecules that are delivered to the mucosal immune system via secretion of outer membrane vesicles (OMVs). We reveal that OMVs require IBD-associated genes, *ATG16L1* and *NOD2*, to activate a non-canonical autophagy pathway during protection from experimental colitis. *ATG16L1*-deficient dendritic cells do not support induction of regulatory T cells ($T_{reg}$) that suppress intestinal inflammation. Immune cells from human subjects with a major risk variant in *ATG16L1* are defective in $T_{reg}$ responses to OMVs. We propose that mutations in IBD-associated genes may contribute to pathogenesis through defects in 'sensing' protective signals from the gut microbiome, defining a novel gene-environment etiology for inflammatory disease.

*SIII-1*

## THE ROLE OF ANAEROBES IN PNEUMONIA: A BREATH OF FRESH AIR?

Jenkins, S.G.*
Weill Cornell Medicine, New York, NY USA

Anaerobic bacteria are well represented amongst the oral microbiota, with numbers ranging from $10^9$/mL in saliva to $10^{12}$/mL in gingival crevices. The ratio of anaerobes to aerobes ranges from 1:1 on teeth to 1000:1 in gingival scrapings. The indigenous oral anaerobic microbiota primarily comprise of *Porphyromonas* and *Prevotella* spp., with *Bacteroides* spp. (non-*B. fragilis* group species) and *Fusobacterium* spp. present in lower numbers. Infections caused by anaerobic bacteria usually result from mucosal barrier breakdown, but several of the oropharyngeal anaerobes display significant pathogenic potential and exhibit numerous pathogenicity factors.

Pleuropulmonary infections are most frequently associated with aspiration of oropharyngeal material by persons with a depressed gag reflex, impaired swallowing, or transient loss of consciousness. Such infections also occur as complications of periodontal disease. Four associated clinical syndromes are described: aspiration pneumonia, lung abscess, empyema, and necrotizing pneumonia. In contrast to the abrupt presentation of acute bacterial pneumonia, aspiration pneumonia follows an indolent course. Patients typically present with chronic pulmonary symptoms and general manifestations of chronic disease, including anemia and weight loss. The affected lung lobes often reflect the patient's position during aspiration.

Although not initially foul-smelling, sputum often becomes malodorous with protracted disease and Gram stains exhibit mixed microbiota. Sputum is an unreliable sample for culture as it is essentially always contaminated with the usual oral bacterial agents harbored by the patient. Cultures of specimens obtained by transthoracic or transtracheal aspiration (albeit rarely employed) may be more valuable. Bronchoalveolar lavage and protected brush specimens may likewise prove valuable, but suffer from the same potential contamination issues as sputa resulting in difficulties ascribing the infection to the specific agents recovered.

Necrotizing pneumonia is characterized by development of numerous micro-abscesses within the pulmonary parenchyma. Lung abscesses most frequently develop in association with periodontal disease, and oropharyngeal anaerobes dominate. Empyema results from protracted anaerobic pulmonary infection with associated malodorous sputum and pleuritic chest pain. Because of the aforementioned difficulties in assessing anaerobic pulmonary infections, they are frequently underdiagnosed and may be inappropriately treated.

## *CUTIBACTERIUM* (EX. *PROPIONIBACTERIUM) ACNES* AND SHOULDER SURGERY

Auwaerter, P.G*.
Sherrilyn and Ken Fisher Professor of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland USA

*Cutibacterium acnes* (*C. acnes*), a microaerophilic anaerobe that grows slowly and forms biofilms, is increasingly recognized as a cause of implant-related infections. Shoulder prostheses appear especially prone to infection with *C. acnes* that in part rests on the skin flora distribution of this organism. Clinical challenges include both diagnostics and treatment. Clinicians often face whether recovery of the organism reflects an authentic infection. While removal of hardware and long-term antibiotic therapy are traditionally performed, it is unclear whether removal is always required and if intravenous therapy yields better outcomes than oral antibiotic regimens. As *C. acnes* accounts for over half of shoulder prosthesis infections, this presentation will review difficulties securing an accurate diagnosis of this infection as well as consider which approaches to treatment lead to best outcomes. Interest in developing novel preventative measures to prevent *C. acnes* biofilm formation is growing.

*SIII-3*

## CHARACTERIZATION OF *CUTIBACTERIUM* (EX. *PROPIONIBACTERIUM*) *ACNES* POPULATIONS IN PATIENTS WITH ACNE VERSUS HEALTHY SKIN—INTEREST OF A LOCAL ADJUNCTIVE THERAPY BASED ON MYRTACINE

Pécastaings, S.;[1] Khammari, A.;[3] Nocera, T.;[4] Peraud, C.;[4] Mengeaud, V.;[5] Dréno, B.;[3] Roques, C.*[1,2]
[1]Fonderephar, Faculté de Pharmacie, Toulouse, France
[2]Laboratoire de Génie Chimique, Université de Toulouse, Toulouse, France
[3]Department of Onco-dermatology, CIC Biotherapy, Nantes, France
[4]Clinical Skin Research Center, Pierre Fabre Dermo-Cosmétique, Toulouse France
[5]Laboratoires Dermatologiques Ducray, Pierre Fabre Dermo-Cosmétique, Cauquillous, Lavaur, France

There has been a paradigm shift in our understanding of the role of C. (*Cutibacterium)* acnes in acne. Instead of resulting from *C. acnes* hyperproliferation, acne would rather result from variations in *C. acnes* population, especially species phylotypes.

Regarding mild acne therapy, topical treatments only are recommended, including antibiotics targeting *C. acnes*. Yet, an increase in antibiotic resistance is observed, especially to erythromycin and clindamycin. With the aim to further understand the role of *C. acnes* and its different phylotypes in acne pathogenesis and resistance, we compared total *C. acnes* populations (including *C. acnes* phylotypes) in acne patients versus healthy subjects. Secondarily, we determined the effect of a cosmetic targeting *C acnes* biofilms in acne patients in a clinical monocentric open exploratory study.

**Methods:** Forehead samplings were performed using Cleaning Strips in 60 patients with mild acne and in 24 healthy subjects (control group). *C. acnes* load was determined by colony forming unit (CFU) counts and erythromycin (EryR) or clindamycin-resistant (ClnR) *Cutibacterium sp* counts. Phylotypes determination of at least 10 clones per patients was made by MALDI-ToF. The clinical evaluation of acne patients (assessment by GEA, porphyrins fluorescence) was done at baseline and after 57 days of treatment (application of a Myrtacine-containing cosmetic twice a day).

**Results:** As a primary objective, we showed (i) similar high levels of *C. acnes* loads in both acne and control groups; (ii) the similar occurrence of EryR and ClnR strains in both groups; (iii) a different repartition of phylotypes in acne patients versus controls with a high prevalence of phylotype IA (higher in acne patients). The link between phylotype IA and erythromycin resistance was also confirmed (iv).

Secondarily, no change in the *C. acnes* load after treatment was observed in acne patients. Nevertheless, a significant decrease of EryR *Cutibacteria* was noted, accompanied with a decrease in the acne severity (GEA) and porphyrins.

**Conclusion:** Myrtacin is efficient *in vitro* on *C. acnes* biofilms alone or combined with antibiotics. Here we show that a myrtacine-based cosmetic could be a potent adjunctive product efficient during the course of acne vulgaris treatment by reducing the level of EryR *C. acnes* counts while acne severity.

*SIV-1*

## EXTRACTING ACTIONABLE KNOWLEDGE FROM THE EHR: MACHINE LEARNING TOOLS FOR PREDICTING RISK OF CDI

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The risk of *Clostridium difficile* infection (CDI) is multifactorial: a patient must be both susceptible and exposed to the pathogen to acquire the disease. In this talk, I will present ongoing work in developing machine learning techniques to build risk stratification models for predicting hospital-onset CDI. Leveraging data extracted from the electronic health record, we model patient risk as a function of the patient's underlying susceptibility and the characteristics of the patient's in-hospital neighbors. This formulation explicitly accounts for the potential of latent spreaders within a hospital setting. Applied to a dataset of in-patient admissions, our model provides actionable insights that could help target interventions and curb the spread of disease.

## MATHEMATICAL MODELING OF *C. DIFFICILE* INFECTION AND ANTIMICROBIAL RESISTANCE

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For healthcare-associated infections, computational and mathematical models of pathogen transmission have become valuable tools to understand sources of transmission and evaluate healthcare interventions, especially in the absence of controlled intervention studies. Among modeling approaches, agent-based models (ABMs) are a popular choice in modeling health-care associated infections. ABMs simulate explicitly each individual in the population. They can incorporate complicated patterns of interaction among individuals in the population, and are able to handle individual variability. Individuals (also called agents) can be characterized by a given number of flexible attributes that influence disease transmission (e.g. antimicrobial use, susceptibility, infectiousness, age, vaccine status, etc.). We developed ABMs of *Clostridium difficile* transmission in hospital wards to identify sources of *C. difficile* infection (CDI) and evaluate control strategies. In this presentation, we will illustrate the assessment of active surveillance and antibiotic stewardship as control strategies to reduce CDI burden and discuss some of the challenges and future perspectives in modeling healthcare-associated infections. Patients with CDI can have three different infection histories; they could be admitted with CDI (CO-CDI), be admitted as colonized patients and became diseased during the hospital stay, or become both colonized and diseased patients during the hospital stay. Screening patients, coupled with isolation precautions for asymptomatic carriers, reduced the number of new colonizations to a greater extent that the number of hospital-acquired CDI cases, which were reduced up to 25%, approximately for scenarios varying diagnostic test characteristics. Antibiotic stewardship interventions had a greater effect in reducing CDI cases than new colonizations. Understanding of the effect of strategies in preventing CDI from different patient histories can lead to implementation of effective bundle interventions by combining synergic and complementary interventions. Additional challenges in modeling healthcare-associated infections include the explicit consideration of interactions among antibiotic use and antibiotic resistant patterns of resistant pathogens, as well as, the integration of transmission models and models of within-host dynamics.


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*SV-1*

## THE DEVELOPMENT OF *CLOSTRIDIUM DIFFICILE* BACTERIOPHAGES FOR THERAPEUTIC PURPOSES

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*Clostridium difficile* is the most common causal agent of bacterial induced infectious diarrhea in the industrialized world. It causes a significant health and financial burden and is difficult to treat as it is only sensitive to three antibiotics. Bacteriophages, or phages are viruses that specifically infect and kill bacteria, and *C. difficile* phages could play a valuable in treating patients infected with this pathogen. We have isolated a large set of *C. difficile* phages with the view to developing them for next generation therapeutics. The most effective phages have been patent protected and shown to target nearly 90% of clinically prevalent and severe strains that circulate in Europe and the USA. Exploitation of phages clearly benefits from a thorough understanding of their biology and much of the research in my laboratory has focused on unraveling how phages interact with *C. difficile* in the natural environment and on determining what dictates a successful phage infection. For therapeutic development it is useful to determine the rates of bacterial resistance to phages compared to the rates of resistance towards antibiotics, and to establish how effective they are at killing *C. difficile* in in vitro, ex-situ and in animal models. In order to better understand how phages interact with *C. difficile* in a gut setting we have optimized ex-situ models to study *C. difficile*-phage interactions on epithelial cells. We have also optimized biofilm, *Galleria mellonella* (insect), and artificial gut models. Data will be presented on how these approaches can inform us of application regimens, dosage and efficacy and of cellular and microbiome responses following cell lysis. Recent data from my laboratory will be presented on these areas.

Jinyu Shan, Ramachandran, A, Thanki, AT, Vukusic, FBI, Barylski, J and **Clokie MRJ.,** 2018. Bacteriophages are more virulent to bacteria with human cells than they are in bacterial culture; insights from HT-29 cells. Scientific Reports. Mar 23, doi: 10.1038/s41598-018-23418-y

Nale JY, Redgwell TA, Millard A, **Clokie MRJ**., 2018. Efficacy of an Optimised Bacteriophage Cocktail to Clear *Clostridium difficile* in a Batch Fermentation Model. Antibiotics. Feb 13;7(1). pii: E13. doi: 10.3390/antibiotics7010013

## ENGINEERING REGULATORY SYSTEMS TO MODULATE GENE EXPRESSION OF *BACTEROIDES* IN THE GUT

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The gut microbiota is implicated in numerous aspects of health and disease, but dissecting these connections is challenging because genetic tools for gut anaerobes are limited. Inducible promoters are particularly valuable tools, because these platforms allow real-time analysis of the contribution of microbiome gene products to community assembly, host physiology, and disease. We developed a panel of tunable expression platforms for the prominent genus *Bacteroides* in which gene expression is controlled by a synthetic inducer. In the absence of inducer, promoter activity is fully repressed; addition of inducer rapidly increases gene expression by 4 to 5 orders of magnitude. Because the inducer is absent in mice and their diets, *Bacteroides* gene expression inside the gut can be modulated by providing the inducer in drinking water. We use this system to measure the dynamic relationship between commensal sialidase activity and liberation of mucosal sialic acid, a receptor and nutrient for pathogens.


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*SVI-1*

## MICROBIOME STUDIES TO DEFINE THE ROLE OF VAGINAL ANAEROBES IN INCREASING SUSCEPTIBILITY TO HIV

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Women in Eastern and Southern Africa carry a disproportionate burden of new HIV infections. Bacterial vaginosis, characterized by a shift in the bacterial biota from mostly lactobacilli to diverse anaerobes is a consistent risk factor. However, specific vaginal bacteria associated with HIV risk are not well defined. Recent investigations have correlated particular vaginal bacterial community types with increased susceptibility to HIV acquisition using molecular methods. In this lecture, we will define high-risk bacteria linked to HIV susceptibility in two studies and highlight the critical need for reproducibility.

## THE VIRTUE OF SIMPLICITY: THE VAGINAL MICROBIOME, GENITAL INFLAMMATION AND HIV RISK

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In a prospective cohort of young, healthy South African women, we found that individuals with diverse genital bacterial communities dominated by anaerobes were at over 4-fold higher risk of acquiring HIV and had increased numbers of activated mucosal CD4+ T cells compared to those with *Lactobacillus crispatus*-dominant communities. We identified specific bacterial taxa linked with reduced (*L. crispatus*) or elevated (*Prevotella, Sneathia*, and other anaerobes) inflammation and HIV infection and found that high-risk bacteria increased numbers of activated genital CD4+ T cells in a murine model. To better understand the dynamics that govern equilibrium states of these cervicovaginal bacterial communities, we analyzed longitudinal samples and constructed a Markov-based model of bacterial community dynamics. We found that while *Lactobacillus crispatus* colonization was relatively stable, *Lactobacillus* iners colonization was unstable with high frequency transition to *Prevotella*-rich, high diversity communities. Overall, this work shows a direct association between the cervicovaginal microbiome and HIV acquisition with community transition probabilities within these African women that favor high risk cervicovaginal communities.

*SVI-3*

## ANAEROBES AND IMMUNITY IN THE FEMALE GENITAL TRACT

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The microbiota colonizing the female genital tract (FGT) have a significant impact on reproductive health, including pregnancy outcomes, fertility, risk of sexually transmitted diseases and post-operative infections. The negative impact of anaerobic infection of the FGT is due in part to the host immune response to these microbes, and associated inflammation. While much attention is focused on the easily accessible vagina and cervix, immune responses in the endometrium and fallopian tubes also play a significant role in reproductive complications related to anaerobic infections. Mucosal immune responses, and the impact of hormonal cycles on these responses, vary throughout the FGT. In the cervix and vagina, proliferation of a diverse anaerobic community is associated with increased numbers and activation of CD4+ T cells, increased concentrations of cytokines and chemokines, but decreased levels of antimicrobial peptides like defensins. In the endometrium, the presence of anaerobes is associated with lower rates of pregnancy implantation. However, low quantity colonization with some anaerobic species is not always associated with endometrial inflammation, suggesting more frequent endometrial trafficking of vaginal microbes than previously thought. This presentation will review host and microbial factors associated with variation in immune responses at all levels of the FGT, and implications for reproductive health outcomes.

***1645*** ***SESSION VII:*** **ORAL PRESENTATIONS I: INTERNATIONAL PERSPECTIVES**

*SVII-1*

## ONGOING RESEARCH ON ANAEROBES IN EUROPE

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There is an increasing interest for anaerobes, not only as causative agents in various infections, but also due to the possibility to identify them directly from the primary isolation plates or from the positive blood culture bottles by the MS-based method. We learn about the changing patterns of the antibiotic susceptibility of these pathogens and with the introduction of the "culturomics" more and more new anaerobic species are described from the normal flora with the hope that their role in health and disease will be investigated later. In Europe the ESCMID Study Group for Anaerobic Infections (ESGAI) has organized several new studies involving expert laboratories from different European countries and beyond.

After 20 years of the first Europe-wide resistance study on Gram negative anaerobes other than *B. fragilis* group, an antibiotic susceptibility surveillance, involving 12 antibiotics, was carried out on *Prevotell*a strains, belonging to 19 different species, collected in 13 countries. Increase in resistance to ampicillin, tetracycline, clindamycin and moxifloxacin was observed and high number of the *P. bivia* isolates, proved to be the most prevalent species, showed multidrug resistance. Two MS systems were also evaluated for the direct identification of *Prevotella* strains. Several country-based evaluations of antibiotic resistance of different anaerobic clinical isolates were published from Europe during the past years, such as Belgium, Croatia, Greece, Hungary, Poland, Slovenia, Spain, Rumania, The Netherlands, and Turkey. Increasing number of reports are dealing with the detection of the multidrug resistance among different anaerobes based on phenotypic and genotypic evaluation. The prevalence of a wide variate of resistance genes has also been evaluated in case of a large collection of clinical and normal flora *Bacteroides* isolates obtained from various European countries and data were compared. Several publications compared the classical identification methods for species identification of anaerobes with the MALDI-TOF MS-based method proving the superiority of the later one for the routine identification of clinically relevant anaerobes directly from the isolation plates. Involving expert laboratories from different European countries, an extensive database development was carried out to improve this capability of the MALDI Biotyper to identify uncommon, fastidious anaerobic species. The largest study ever, involving 5300 *B. fragilis* clinical isolates, proved the applicability of the MALDI-TOF MS method for timely detection of isolates, which belong to Division II and harbor the *cfi*A (carbapenemase) gene, helping with this the empirical selection of antibiotic treatment in case of severe infection caused by *B. fragilis*. Similar evaluation is in progress to apply the high molecular weight typing of *C. difficile* isolates by MALDI-TOF MS. By changing the methods used in routine laboratories for diagnosing anaerobic infections, the need to have an external quality control scheme is needed. Two study groups of ESCMID (ESGAI and ESCMID Study Group for Genomic and Molecular Diagnostics - ESGMD) together with expert laboratories in Europe are working on to set up International Anaerobe Quality Assurance Schemes to prove the level of anaerobe diagnostics in routine laboratories throughout Europe.

# UK *BACTEROIDES SPECIES* SURVEILLANCE SURVEY: CHANGE IN ANTIMICROBIAL RESISTANCE OVER 16 YEARS

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The prevalence of antimicrobial resistance in unselected clinical isolates of *Bacteroides* spp. was determined in a UK-wide surveillance survey in 2016 and compared to a similar cohort from 2000.

Important components of the human gut microbiome, *Bacteroides* spp. are an important cause of opportunistic infection. Rising rates of resistance reported across Europe, together with increasing referrals of resistant *Bacteroides* spp. to the UK ARU prompted this study.

UK clinical microbiology laboratories were invited to submit sequential isolates of *Bacteroides* spp. from any clinical specimen. After de-duplication, 168 isolates were analysed from 14 laboratories in 2016, and 224 from 2000. Identification was confirmed by MALDI-TOF MS, and partial 16S DNA sequencing. Isolates were tested by agar dilution to metronidazole, clindamycin , co-amoxiclav, pip-tazobactam, meropenem, moxifloxacin, tigecycline, chloramphenicol and linezolid. Phenotypic resistance was correlated with genetic mechanisms of resistance including *nim*, *cfiA* and *erm* genes.

*B.fragilis* comprised 69% of isolates in 2000 and 77% in 2016. An overall increase in reduced susceptibility was seen across all commonly used antimicrobials from 2000 to 2016. The greatest rate of resistance was in clindamycin, with 23.8% of isolates having reduced susceptibility in 2016 vs 12.5% in 2000. 4 metronidazole resistant isolates were present in 2016 (2.4%), a 6 fold increase. A significant rise in reduced susceptibility to co-amoxiclav was seen (8.5% -17.9%) with smaller rises in pip-tazobactam (5.7-8.4%) and meropenem (2.7-6.0%). Rising rates of resistance to moxifloxacin (7.2-16.7%) and tigecycline (5.4-13.1%) were seen, whereas linezoid and chloramphenicol retained high rates of susceptibility. Local geographical variation in resistance was observed.

This is the first UK *Bacteroides spp*. antimicrobial resistance surveillance survey. Significant rises in resistance across several classes of antimicrobials makes susceptibility testing of important clinical isolates paramount. Monitoring and surveillance of resistance trends at regular intervals is imperative.

*SVII-3*

## *CLOSTRIDIOIDES DIFFICILE*: WHAT DO WE KNOW ABOUT THIS PATHOGEN IN BRAZIL?

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*Clostridioides difficile* is the leading etiologic agent associated with nosocomial diarrhea in adults with a compromised gut microbiota, caused by the use of broad-spectrum antibiotics. The infection caused by *C. difficile* (CDI) is a multi-factorial process, which includes the disruption of the intestinal microbiome, acquisition, germination and outgrowth of spores and the colonization of the intestinal tract. The main symptoms related to the CDI include severe episodes of diarrhea, which can progress to a pseudomembranous colitis and toxic megacolon. The severity of the disease is correlated to *C. difficile* strains that produce of toxins (*TcdA* and *TcdB*), in different levels. The epidemiology of this pathogen is mainly based on a PCR ribotyping, which gives a global analysis of *C. difficile* related virulent strains based on a reference library. Thus, several ribotypes have been reported in Europe and in the United States, specifically the epidemic the strain NAP1/027, 001 and 014, which caused an increase in the incidence of the CDI and mortality rates. While in Latin American countries (Chile, Panama, Colombia and Costa Rica) the ribotype 027 was reported, in Brazil no isolation was described, up to now. Nevertheless, other ribotypes have been isolated, such as, 010, 020, 133, 135 and 233, from hospital environment, human CDI cases and domestic and wild animals. Two of them, 133 and 135 were only reported and circulate in Brazil. In the past 20 years, we have been characterizing our strains at a microbiological level and by using genotypic tests, and more recently the whole genome sequence (WGS) and proteomics to gather information about the circulation and virulence factors of the Brazilian ribotypes. One of the main difficulties faced in Latin American countries is the fact that ribotyping is not performed here, so we have been developing an advanced mass spectrometry technic to directly "ribotype" strains through MALDI-TOF. Mostly, our results have been revealing new *C. difficile* ribotypes and a high variety profile of strains circulating in our country.

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# NEW INSIGHTS INTO *CLOSTRIDIUM DIFFICILE* (CD) INFECTION IN LATIN AMERICA: NOVEL DESCRIPTION OF TOXIGENIC PROFILES OF DIARRHEA-ASSOCIATED CD IN BOGOTÁ, COLOMBIA

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*Clostridium difficile* (CD) produces antibiotic associated diarrhea and leads to a broad range of diseases. The source of CD infection (CDI) acquisition and toxigenic profile are factors determining the impact of CD. This study aimed at detecting healthcare facility onset- (HCFO) and community-onset (CO) CDI and describing their toxigenic profiles in Bogotá, Colombia. 217 fecal samples from patients suffering diarrhea were simultaneously subjected to two CDI detection strategies: i) *in vitro* culture using selective chromogenic medium (SCM), followed by verification by colony screening (VCS), and ii) molecular detection targeting constitutive genes, using two conventional PCR tests (conv.PCR) (conv.16S y conv.gdh) and a quantitative type of test (qPCR.16s). The CD toxigenic profile identified by any molecular test was described using independently 6 tests for describing *PaLoc* and *CdtLoc* organization. High overall CDI frequencies were found by both SCM (52.1%) and conv.PCR (45.6% for conv.16S and 42.4% for conv.gdh), compared to reductions of up to half the frequency by VCS (27.2%) or qPCR.16S (22.6%). Infection frequencies were higher for SCM and conv.16S regarding HCFO but greater for CO concerning conv.gdh (with statistically significant differences). Heterogeneous toxigenic profiles were found, including amplification with lok1/3 primers simultaneously with other *PaLoc* markers (*tcdA*, *tcdB* or *tcdC*). These findings are the first report regarding the differential detection of CDI using *in vitro* culture and molecular detection tests in Colombia and the circulation of CD having heterogeneous toxigenic profiles which could affect the impact of CDI epidemiology.


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***0745*** ***SESSION VIII:*** **ORAL PRESENTATIONS II: SEQUENCING & PROBIOTICS**

*SVIII-1*

## CHALLENGES OF NEXT-GENERATION-SEQUENCING TARGETING ANAEROBES

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Next-generation sequencing is the ultimate tool for analyses of microbiomes and metagenomes associated with infection (clinical microbiology) or dysbiosis (microbial ecology) but also for bacterial molecular typing (e.g. outbreak-associated). The main technologies are Illumina short read sequencing, Pacific Biosciences and Oxford Nanopore long read sequencing; the latter distinguished because of the miniaturization potential. In this talk, the newest developments will be presented and discussed in the light of anaerobes and anaerobic infection/dysbiosis.

A pre-condition for a representative result is the quantitative lyses of cells. Some of the anaerobes such as Clostridia are robust while others of the same environment, such as spirochetes, possess a very thin murein sacculus. The lysis efficiency of mechanical versus chemical and combinatory procedures will be discussed and recommendations given. For Illumina short read sequencing a primer pair not amplifying more than 520 bp should be selected to avoid ambiguities. Therefore, in most studies the V34 or V345 16S rRNA-gene region is selected but members of classes Clostridia and Spirochaetes are under-represented in the resulting profiles. Vice versus V123 directed primers miss some Synergistia and Archaea. For a complete microbiome analysis in mixed anaerobic infections and environments long read sequencing is a *conditio sine qua non*.

# PHYLOGENOMIC ANALYSIS OF *FUSOBACTERIUM NECROPHORUM* BASED ON WHOLE GENOME SEQUENCING AND ITS ASSCOCIATION WITH DISEASE AND HOST

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**Objective:** *Fusobacterium necrophorum* (FN) is the main cause of Lemierre's syndrome and FN has also been associated with tonsillitis and peritonsillar abscesses, otitis media and colorectal cancer. FN may also be found in asymptomatic carriers. Virtually nothing is known about this bacterium at the molecular and genetic level. The aim of this study was by whole genome sequencing of a large volume of strains isolated from patients with different diseases and hosts to determinate the phylogenetic diversity of FN and to identify whether certain phylogenetic lineages of FN may be associated with specific diseases.

**Material and Methods:** In total, genomes of 85 strains were analyzed. Of these, 70 were from our own strain collection while the genomes of 15 strains were extracted from Genbank. Phylogenomic analysis were based on single nucleotide polymorphism (SNP) analysis using the software program parsnp while pairwise average nucleotide identity (ANI) was calculated in the JSpecies program.

**Results:** Phylogenomic analysis based on SNPs of the whole genome sequences revealed that the FN strains grouped into three distinctive clades, corresponding to the two subspecies of FN. The third clade consisted of two penicillin-resistant human isolates of FN. ANI calculations showed that the interclades identity were around 95% supporting separation of FN into subspecies. Strains of *F. necrophorum* subsp. *funduliforme* clustered into two distinct clusters. Within both cluster a clonal relationship between most of the strains were observed**.** Most interestingly, no separation of the strains based on the host or disease could be found.

**Conclusions:** No lineages of FN were associated with specific diseases in humans and invasive strains were phylogenetically similar to strains from local infections and strains isolated from healthy carriers. Also, human isolates of FN were similar to animal strains. Our results indicate that virulence and invasiveness of FN is not associated with a specific phylogenetic lineage. Therefore, FN infections might involve additional factors to cause infections (host specific and other external factors (e.g. viruses)). Preliminary data on the intracluster virulence potential and its relationship to disease will be presented at the congress.

*SVIII-3*

## *LACTOBACILLUS* COCKTAILS MODULATE MONOCYTE IMMUNE RESPONSES TO PATHOGENIC VAGINAL ANAEROBES

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A disruption to the normal vaginal biome commonly diagnosed as bacterial vaginosis (BV) allowing for an overgrowth of pathogenic vaginal anaerobes has been positively correlated with inflammation and an increased susceptibility to sexually transmitted infections (STIs) e.g. HIV, HSV, chlamydia, gonorrhea and trichomoniasis. *G. vaginalis* (GV) and *P. bivia* (PB) are being considered as signature BV bacteria that act synergistically with other STI pathogens to break down the vaginal immune barrier. The presence of certain *Lactobacillus* species has shown to mediate aspects of the vaginal biome disruption; however, not all *Lactobacillus* species or strains within a species are equally beneficial. As a defense against a disrupted vaginal biome, we have developed multiple iterations of a live cocktail of *Lactobacillus* strains representing species most consistently associated with vaginal health. We tested the ability of the *Lactobacillus* species alone and cocktailed together to modulate monocyte immune responses to a BV bacteria-colonized vaginal epithelium in an *in-vitro* model. We applied a novel high-fidelity quantitative nuclease protection assay coupled with NextGen sequencing to interrogate over 2000 immunology and cell signaling genes. When applied as a monoxenic probe, two *Lactobacillus* species induced differential gene expression by immune-stimulated bystander human monocytes. Among the different *Lactobacillus* cocktails tested, one iteration induced a robust differential gene expression pattern, with a significant number of uniquely modified gene expression levels (78) as compared to other iterations of the cocktail and the monoxenic probes (up to 20). The *Lactobacillus* cocktails significantly modified gene responses to both GV and PB. Among the genes most significantly upregulated by both GV and PB and downregulated in the context of *Lactobacillus* colonization were IDH1 and FCER2, both of which are associated with HIV transmission. Other potential novel targets for therapeutic interventions are being indentified.

# *CLOSTRIDIUM DIFFICILE* VIRULENCE FACTORS IMPAIRED BY BIO-K+ PROBIOTICS

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*Clostridium difficile* infections (CDI) result from antibiotic use and cause severe diarrhea which is life threatening and costly. The Centers for Disease Control and Prevention seek an action to prevent this drug-resistant infection in hospital settings. *Lactobacilli* employ non-specific mechanisms, such as secretion of organic acids, bacteriocins or hydrogen peroxide, to compete with pathogens like *Clostridium difficile* (CD). Yet many *Lactobacilli*-based probiotics have failed to demonstrate efficacy in preventing CDI. The probiotic containing *L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 is the only probiotic product licensed by Health Canada to reduce the risk of CDI. In clinical trials, hospitalized adults administered with Bio-K probiotics alongside antibiotics had fewer cases of antibiotic-associated diarrhea and CDI than placebo. Previously, this probiotic has demonstrated a strong inhibitory effect on the growth of several nosocomial CD strains by production of antimicrobial metabolites during fermentation. Here we have investigated a novel mechanism that these strains use to impair CD virulence. In a first set of experiments, the hypervirulent strain CD R20291 was co-cultured anaerobically with these probiotic strains in RCM broth for 48h at 37ºC. In a second series, CD R20291 was co-cultured under the previous conditions, but in TY broth in order to stimulate CD toxin production while preventing lactic acid (LA) acidification. In RCM broth, after 12h of incubation there was a rapid decline in pH which correlated with an inhibition of CD growth. In TY broth, CD growth was normal, but toxin A/B secretion was significantly reduced by more than 90% at 24h. This effect was maintained through 48h. These results suggest that the specific probiotic containing *L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 interferes with CD pathogenesis by: 1) inhibiting growth via LA secretion and, 2) reducing toxin A/B synthesis without acidification. This concludes that the production of organic acids is not the only mechanism responsible of the CD toxicity inhibition.


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***0900*** ***SESSION IX:*** **THE ORAL MICROBIOME & HUMAN HEALTH**

*SIX-1*

## *P. GINGIVALIS* AS AN ORCHESTRATOR OF DYSBIOSIS IN PERIODONTITIS

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Recent advances from microbiome and host response mechanistic studies indicate that periodontitis is not a bacterial infection in the classical sense but rather results from dysbiosis owing to a breakdown in host-microbe homeostasis. Accordingly, periodontitis is induced in susceptible hosts by a polymicrobial community, in which different members have distinct roles that converge synergistically to enhance colonization, nutrient procurement and persistence in an inflammatory environment. *P. gingivalis*, a gram-negative anaerobic bacterium that has long been associated with human periodontitis, was shown to manipulate the host immune response and remodel a symbiotic community into a dysbiotic one. *P. gingivalis* thus acts as a keystone pathogen whose effects have community-wide significance and are disproportionate of its abundance. In general, keystone pathogens — the colonization of which is facilitated by accessory pathogens — initially subvert the host response leading to the emergence of a dysbiotic microbiota, in which pathobionts overactivate the inflammatory response and cause tissue destruction. Periodontal inflammation and dysbiosis positively reinforce each other because inflammatory tissue breakdown products are used as nutrients by the dysbiotic microbiota, thereby contributing to its persistence and chronic inflammation. These concepts have important implications for novel therapeutic approaches, including strategies for microbial community manipulation and targeted modulation of the host response to limit destructive inflammation and reverse the microbial immune subversive tactics that fuel dysbiosis.

## TARGETING OF THE IRON-DEPENDENT METABOLISM OF ORAL ANAEROBIC BACTERIA

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While the inflammatory conditions associated with periodontal diseases (PD) are triggered by bacteria, not all bacteria are pro-inflammatory and indeed, some bacteria are beneficial as they provide a protective barrier against PD. Here we investigated a novel systemic therapeutic, amixicile that selectively targets the pyruvate: ferredoxin oxidoreductase (PFOR) found in the pathogenic anaerobic bacteria associated with PD. Bioinformatic analysis of the genomes of bacteria deposited in the Human Oral Microbiome Database (HOMD) revealed that the iron-dependent enzyme, PFOR is present in many anaerobic bacteria implicated in development and progression of PD. Such findings indicate that amixicile would inhibit growth of most of the pathogens. To verify its effectiveness bacteria were grown in broth and biofilm forms in both monocultures and polymicrobial mixtures in the presence and absence of amixicile. Growth of broth monocultures of most of the PFOR-coding bacteria (*Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Prevotella intermedia*) was inhibited by amixicile while growth of the commensal bacterium, *Streptococcus gordonii*, containing pyruvate dehydrogenase was unaffected by amixicile. Also amixicile at concentration of 5µg/ml completely inhibited growth of *P. gingivalis, P. intermedia*, and *F. nucleatum* grown in biofilm. Furthermore, this activity was specific as no inhibition was observed when biofilm-grown *S. gordonii* was incubated in the presence of amixicile. Finally, amixicile was effective on PFOR – containing bacteria grown in multispecies biofilms as growth of *P. gingivalis, P. intermedia, T. forsythia*, and *F. nucleatum* was significantly inhibited while growth of *S. gordonii* and *A. actinomycetemcomitans* was unaffected. The effect of amixicile on the complex human oral microbiome was tested using a human oral subgingival microbiome derived from human subjects with periodontal disease. Our data showed that in such a complex system amixicile selectively inhibits growth of anaerobic bacteria and transitions microbiome predominant in Bacteroidales (35%) to one dominant in Lactobacillales [*Streptococcus* spp – 59% and *Lactobacillus* spp – 27%]. Such treated microbiome had a lower pro-inflammatory effect on Human Oral Keratinocytes (HOKs) when compared with untreated microbiome. Overall, our results show that amixicile selectively inhibits growth of anaerobic pathogenic bacteria associated with PD while leaving health promoting bacteria unaffected thus reducing the pro-inflammatory nature of the dysbiotic microbiome. Such therapeutic has a great promise for a targeted therapy for PD.

*SIX-3*

## SYNGENIC DNA: MAKING EVERY BACTERIAL SPECIES GENETICALLY TRACTABLE

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Genetic engineering is a powerful approach for discovering fundamental aspects of bacterial physiology, metabolism, and pathogenesis. The problem is the vast majority of bacteria that can be grown in a laboratory remain genetically intractable, beyond the power of genetics for elucidating function or for engineering for human use. The challenge of genetic intractability stymies basic-, synthetic-, and translational-microbiology research and development. Researchers spend years constructing ad hoc genetic systems one species at a time, an arduous and expensive process. But what if every bacterium that could be grown in the laboratory could be quickly and easily made tractable? How rapidly would microbial research progress if every bacterial strain was as genetically accessible as commercially available *E. coli*?

Here, we introduce SyngenicDNA, a method for rendering cultivable bacterial species genetically tractable irrespective of their taxonomic lineage or genetic barriers. The approach takes advantage of state-of-the-art combinatory genome and epigenome Single-Molecule-Real-Time (SMRT) sequencing technology. It has been designed to prevent non-self DNA (genetic tool) degradation by innate Restriction Modification (RM) and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Cas systems; underlying causes of genetic intractability that exist within most bacteria. SyngenicDNA overcomes these complex bacterial defense mechanisms using a rapid and widely applicable "stealth" based strategy.

The paucity of genetically tractable bacteria is a formidable challenge to deciphering functional attributes of members of the human microbiome. Thus, as proof of principle, we are demonstrating the power of the SyngenicDNA method on bacterial species from the human oral microbiota. We intend to create an initial repository of >100 model bacterial strains representing anaerobic and aerobic species across eight different phyla within the oral microbiota, each made genetically tractable using the SyngenicDNA method. Our overarching goal is to provide universally applicable methodologies to rapidly render every bacterial species genetically tractable.

# NITROSATIVE STRESS SENSING IN *PORPHYROMONAS GINGIVALIS*: STRUCTURE AND FUNCTION OF THE HEME BINDING TRANSCRIPTIONAL REGULATOR HcpR

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*Porphyromonas gingivalis* is a Gram negative anaerobe implicated in the progression of periodontal disease. It is capable of surviving in the high levels of reactive nitrogen species found in the oral cavity due to its efficient nitrosative stress response. HcpR is an important sensor-regulator that is vital in the initiation of the nitrosative stress response in many Gram negative anaerobic bacteria. Knockout of the *hcpR* gene in *P. gingivalis* results in the inability of the bacteria to grow in physiological concentrations of nitrite and complementation of *hcpR* using the novel plasmid Pg108 rescues this phenotype. HcpR causes a dose dependent upregulation of a gene coding for a putative NO reductase when exposed to nitrite or nitric oxide. Full transcriptome sequencing reveals that *hcp* is the most significantly upregulated gene when *P. gingivalis* is exposed to nitrite and knockout of *hcp* resulted in a phenotype that is similar to that of the *hcpR* deficient strain. HcpR regulates the expression of *hcp* via direct binding to an inverted repeat sequence in the promoter region of the *hcp* gene. We present a 2.6 Å crystal structure of the N-terminal sensing domain of HcpR and show that it is FNR-CRP regulator. A putative hydrophobic heme binding pocket was identified in the junction between the N-terminal domain and the dimerization helix. Mutation of two methionine residues (Met68 and Met145) in this pocket abrogates activation of HcpR thus verifying the binding site. Heme bound to HcpR exhibits heme iron as a hexa-coordinate system in the absence of nitric oxide (NO) and upon nitrosylation transitions to a penta-coordinated system. Finally, SAXS experiments of the full length HcpR reveal that the C-terminal DNA binding domain of HcpR has a high degree of interdomain flexibility. In conclusion, HcpR is a heme binding FNR-CRP transcriptional regulator that plays an irreplaceable role in the sensing of reactive nitrogen species and regulation of genes necessary for their detoxification.


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*SX-1*

## NEW INSIGHTS INTO *FUSOBACTERIUM*-ASSOCIATED COLORECTAL CANCERS

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In colorectal cancer (CRC), malignant cells are surrounded by a complex microenvironment encompassing a range of non-transformed cells, but also a diverse collection of microorganisms. A growing body of evidence demonstrates the role of particular microorganisms in modulating inflammatory environments and promoting tumor growth and metastasis. Studies by our group, and others, reveal a consistent enrichment of *Fusobacterium nucleatum* in human CRC, and *F. nucleatum* has been shown to accelerate tumorigenesis using both *in vitro* and *in vivo* preclinical models.

We demonstrate via microbiome analysis and microbial culture that fusobacteria and its co-occurring microbiota, including *Bacteroides, Prevotella* and *Selenomonas* species, persist in liver metastasis of *Fusobacterium*-positive CRC. Many of the liver metastasis share the same dominant (>1% relative abundance) microbiome as the paired primary CRC tumors. Additionally, we have cultured *Fusobacterium* sp. from paired primary and metastatic tumors, and via whole genome sequencing analysis reveal the same strains of *Fusobacterium* are present in the primary tumors and distant site metastasis, despite the tissue being resected months or even years apart. In situ hybridization analysis demonstrate that *Fusobacterium* is invasive in the primary tumors and distal metastasis, and is associated with malignant cells.

Additionally, we demonstrate via microbiome analysis and microbial culture, that *Fusobacterium* and its co-occurring microbiome also persist and remain viable in patient derived xenografts of CRC. Treatment of a patent derived colon cancer xenograft harboring *Fusobacterium*, with an antibiotic that kills *Fusobacterium*, reduced tumor growth, cancer cell proliferation and tumor fusobacterial load.

We have isolated and sequenced 80 *F. nucleatum* strains from human CRC with detailed microbiome and patient metadata. In addition to phenotypic analysis and small molecule inhibitory screens of the *F. nucleatum* CRC isolates, we are conducting comparative genomic analysis with *F. nucleatum* isolates from the oral cavity of non-cancer patients to determine colorectal cancer specific markers.

These findings suggest that the tumor microbiota are intrinsic and essential components of the cancer microenvironment and warrants further investigation into the modulation of the tumor microbiota for the treatment of *Fusobacterium*-associated CRC in both early and late stage disease.

# *FUSOBACTERIUM* IN THE ORAL CAVITY AND ITS SIGNALING POTENTIAL

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The aim of this study was to investigate the role of the different subspecies of *Fusobacterium nucleatum* in health and disease associated biofilms in the oral cavity.

*F. nucleatum* is an anaerobic species primarily found in the dental plaque biofilm, where it is seen as a key player in the emergence of a dysbiotic microflora in periodontitis. However, *F. nucleatum* has also been associated with non-oral diseases such as atherogenic cardiovascular disease, rheumatoid arthritis, inflammatory bowel disease and colorectal cancer.

Plaque biofilms consist of multiple different microorganisms, aggregating in an organised manner. While this aggregation of different species is well documented, their means of communication and in particular their mechanisms of recruitment are less well understood. The role of *F. nucleatum* is of particular interest as it interacts with multiple species in the oral biofilm and modulates progression from health to disease. Its role in controlling oral health could be pivotal.

Comparative genomics were employed to analyse the five currently described *F. nucleatum* subspecies. The genomic variation between the strains will be presented and differences correlated to observed phenotypes, including coaggregation, signalling capabilities and metabolism.

*Fusobacterium nucleatum* is an opportunistic human pathogen with pivotal importance in the formation of a disease-associated biofilm in periodontitis. Currently five subspecies (*nucleatum, polymorphum, vincentii, animalis* and *fusiforme*) have been described. Here we report phenotypic and genotypic differences between those and highlight potential implications for health and disease.

*SX-3*

## ORAL BACTERIA AND CRC: WHAT IS THEIR ROLE?

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Multiple studies have now demonstrated an enrichment of oral bacteria including *Fusobacterium nucleatum*, *Gemella morbillorum*, and *Parvimonas micra* in colorectal cancer patients compared to healthy controls. This talk will discuss ongoing studies in the Sears laboratory regarding the potential role of oral microbes in colon tumorigenesis at both the single species and consortium level, including colonic biofilms.

# *FUSOBACTERIUM NUCLEATUM* AND ADVERSE PREGNANCY OUTCOMES: A REVIEW OF EPIDEMIOLOGICAL AND MECHANISTIC EVIDENCE

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*Fusobacterium nucleatum* is a Gram-negative anaerobic oral commensal commonly found in periodontal disease. *F. nucleatum* has been associated with multiple systemic diseases, including oral, gastro-intestinal, rheumatologic, and vascular pathology. As pregnancy is associated with an increased risk of periodontal disease, there has also been significant research into the effects of periodontal disease on adverse pregnancy outcomes. This article reviews the epidemiological and mechanistic evidence of the association and role of *F. nucleatum* in adverse pregnancy outcomes.

**Key Words**: oral anaerobe, pregnancy complications, preterm birth, stillbirth, neonatal sepsis, VE-cadherin, placenta, chorioamnionitis, *Fusobacterium nucleatum*, FadA.


WELCOME
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***1400*** ***SESSION XI:*** **DOES THE HUMAN MICROBIOME IMPACT TREATMENT OF NON-INFECTIOUS DISEASE**

*SXI-1*

## THE GUT MICROBIOME AFFECTS THE UPTAKE AND METABOLISM OF DRUGS

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Understanding how drugs are absorbed and metabolized before reaching their site of action is critical for accurate dosing to maximize efficacy and minimize the risks of adverse drug response. Our group and others have established that gut microbes can have direct impacts on a wide range of xenobiotics; however, mechanistic understanding of microbial effectors is key to the creation of actionable microbiome-based diagnostics and treatments. To facilitate these studies, we created a culture and genome collection of gut Actinobacteria, focusing on the prevalent organism *Eggerthella lenta*, to mine for effectors of xenobiotic metabolism. Phenotype-screening, comparative genomics, and heterologous expression have uncovered multiple loci involved in xenobiotic interactions including an 8-gene cluster directly responsible for the metabolism of cardenolides including the cardiac medication digoxin. In addition to direct metabolism of drugs, we have also identified that particular members of the microbiome, including *E. lenta*, can modulate both expression and function of the broad specificity host drug-transporter *p*-glycoprotein (PGP). PGP functions to prevent the absorption of many xenobiotics from the GI tract, including digoxin. Through *in vitro* models and pharmacokinetic analyses in gnotobiotic mice with defined microbiota compositions, we have established that modulation of drug transport is a community-composition specific trait. We have observed a 10-member community of prevalent gut microbes results in transcriptional induction of PGP which is concomitant with decreased drug absorption. Alternatively, mono-colonization with *E. lenta* results in transcriptional induction but increased drug absorption. Cell culture models have revealed *E. lenta*-derived small molecule(s) capable of direct transport-inhibition resulting in compensatory transcriptional induction. These analyses highlight a complex and dynamic interplay between host, microbiota, and xenobiotics. Continued progress in this area promises new microbiome-based diagnostics and therapies to enhance and predict drug response.

## THE CONTRIBUTION OF ORAL ANAEROBES TO HYPERTENSION: IS THERE NO LINK?

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Having high blood pressure puts you at risk for heart disease and stroke, which are leading causes of death in the United States and worldwide. One out of every 3 Americans has hypertension and it is estimated that despite aggressive treatment with medications, only about half of those medicated have managed blood pressure. Recent discoveries of the oral microbiome that reduce inorganic nitrate to nitrite and nitric oxide provide a new therapeutic target for the management of hypertension. The presence or absence of select and specific bacteria may determine steady state blood pressure levels. Eradication of oral bacteria through anti-septic mouthwash or over use of antibiotics causes blood pressure to increase. Allowing recolonization of nitrate and nitrite reducing bacteria can normalize blood pressure. This presentation will provide evidence of the link between oral microbiota and the production of nitric oxide and regulation of systemic blood pressure. Management of systemic hypertension through maintenance of the oral microbiome is a completely new paradigm in cardiovascular medicine

*SXI-3*

## ANAEROBES AND THE BRAIN: THE ROLE OF COMMENSAL MICROBIOTA IN NEUROLOGIC DISEASE

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The intestinal microbiota plays a substantial role in immunologic and neurologic development and is crucial for maintaining lifelong homeostatic function. Recently evidence suggests that the microbiota contributes to neurodegenerative diseases, including multiple sclerosis, Alzheimer's disease, Parkinson's disease, and amylotrophic lateral sclerosis. Because of immature immune function in infancy as well as immunosenescence in the elderly, both early- and late-life represent windows in which people may be more vulnerable to microbiota alterations, and this may account for the initiation or progression of age-related metabolic and neurologic diseases. In the elderly, age-related changes in the gut microbiota contribute to immune dysfunction in aging. We now show that mice genetically susceptible to Alzheimer's disease have accelerated changes in their aging gut microbiota, which can be rescued via a calorie-restriction diet with 30% less calories from carbohydrates. This dietary modification also prevents amyloid-beta plaque accumulation in female mice and enriches protective microbiota in a sex-dependent manner. These data suggest that manipulating the aging microbiota through dietary modification may be a potential therapeutic strategy to prevent Alzheimer's disease, and potentially provides additional microbial targets to promote lifelong neurologic health.

*1540* ***SESSION XII:*** **TAXONOMY CHANGES AND THE CLINICAL IMPLICATIONS**

*SXII-1*

## WINDS OF CHANGE: TAXONOMIC RESTRUCTURING OF THE CLINICALLY IMPORTANT CLOSTRIDIA

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In 1994, Collins and colleagues used 16S rRNA gene sequencing to reveal the considerable phylogenetic diversity present within the genus *Clostridium*. Nineteen distinct phylogenetic clusters were defined and suggested that cluster I be reserved for the 'true' representatives of the genus. But, it was not until 2016, that Lawson and Rainey formally proposed that the genus *Clostridium* should be restricted to the type species *Clostridium butyricum* and close relatives within the clostridial cluster I and be recognized as *Clostridium sensu stricto*. Although many species of the genus that fall outside rRNA cluster I have been reclassified to novel taxa, many organisms still retain the designation *Clostridium* and therefore the taxonomic status of this genus is still in a state of flux; many species that are phylogenetically located outside *Clostridium sensu stricto* require reclassification. The majority of these changes do not impact the clinical community, but as seen recently with the reclassification of *Clostridium difficile* to *Clostridioides difficile,* such changes can have major implications. The presentation will highlight other clinically important *Clostridium* species that have undergone, or require taxonomic revision.

## CLINICAL IMPLICATIONS OF ANAEROBIC TAXONOMY CHANGES

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The explosion in sophisticated molecular tools and the characterization of the human microbiome have resulted in the discovery of a variety of novel organisms. As we add knowledge to the expanding world of microbial diversity, reclassification of established species into new taxa is a natural consequence. Reclassification is useful for understanding evolutionary biology, interactions between microbes, pathogenesis, and the epidemiology of infections. However, for the clinical laboratory, significant changes to established nomenclature, such as reassignment of *Clostridium difficile* into the new genus *Clostridioides difficile* and *Propionibacterium acnes* into the new genus *Cutibacterium acnes* creates some challenges. This session will present some practical implications of anaerobic taxonomy changes. Participants are invited to share their experiences as well.


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***1630*** ***SESSION XIII:*** **ORAL PRESENTATIONS III: *CLOSTRIDIOIDES (CLOSTRIDIUM) DIFFICILE*—LABORATORY RESEARCH**

*SXIII-1*

## EXTRACELLULAR VESICLES PRODUCED BY *CLOSTRIDIOIDES DIFFICILE*

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Extracellular vesicles are important cellular products capable of carrying many types of concentrated molecules, including LPS, toxins, DNA and bacterial virulence factors, and protect them from the environment. In Gram negative bacteria, these structures originate in the outer membrane and have been studied in detail. Although gram positive bacteria do not possess an outer membrane, vesicles originated in the cytoplasmic membrane have been described. In the opportunistic pathogen *Clostridioides difficile*, a Gram positive bacteria, toxigenic and with high levels of antibiotic resistance, thess structures havs been detected only recently. In this study, the extraction and purification of the extracellular vesicles (EVs) were performed by ultrafiltration of the supernatant of a stationary phase culture followed by differential centrifugation in a density gradienn. The fractions obtained were analyzed by transmission electron microscopy (TEM) and scanning electron microscopy (SEMy. polyacrylamide gel electrophoresis was employed to verify the protein pattern of the MVs fractionse EV enriched fractions were analyzed by mass spectrometry and commercial ELISA assays were used to detect the presence of toxin A and B. We obtained TEM images of purified vesicles with sizes ranging between 70 and 500 nm. According to dynamic light scattering analysis the average size of the vesicles was 150 nm. In addition, SEM and TEM images showed that the vesicles are released in the terminal and sub-terminal location of the cell, in the region of the bipartition site. *C. difficile* toxins, especially B toxin, was present in the EV material. The identification of the proteins and cellular components segregated in EVs will help us understand important steps in the pathogenesis of *C. difficile*, EVs might also be useful for vaccine development since previously studies utilized that structure as a possible inductor of the immune response.

**Key-words:** *Clostridioides difficile*, Extracellular Vesicles, Electron microscopy

# CHARACTERIZATION OF GERMINATION INHIBITORS AGAINST *CLOSTRIDIUM DIFFICLE* R20291

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*Clostridium difficile* infections (CDI) are the leading cause of hospital-acquired diarrhea worldwide. Under normal circumstances, bacteria found naturally in the gastrointestinal tract are able to provide a barrier against *C. difficile* colonization. Upon antibiotic therapy, the protective barrier is lost as the microbial community becomes depleted—this then provides the opportunity for *C. difficile* to colonize the human gut. Exposure to taurocholate, a natural bile salt found in the gastrointestinal tract, causes *C. difficile* spores to begin their transition, a process known as germination, from metabolically dormant structures to toxin-producing cells. As germination is required for CDI, anti-germination compounds can serve as disease prophylaxis.

While the taurocholate receptor is currently unknown, chemical probes can be used to determine structure-activity relationships; these analyses can allow for the rational design and development of potent anti-germination drug candidates. CAmSA, a synthetic analog of taurocholate, was previously shown to inhibit *C. difficile* 630, a laboratory type strain, *in vitro* with an IC50 of 58.3μM. CAmSA was also shown to protect mice from CDI by inhibiting spore germination *in vivo*.

CAmSA, however, shows no activity against R20291, a hypervirulent strain of *C. difficile*. In this study, a library of 134 CAmSA analogs was synthesized and the efficacy of each analog was determined against *C. difficile* R20291 spores. Our structure-activity relationships suggest that shorter alkyl linkages between the amide and the aromatic sidechain, as well as a more constrained ring structure, allow for better inhibition. We have identified 13 anti-germinants with IC50 values below 50μM. Of these anti-germinants, we report an analog that is 30 times more potent against R20291, than CAmSA was against 630. Several CAmSA analogs identified in this study account for some of the most potent anti-germinants reported so far against *C. difficile*.

*SXIII-3*

## PERSISTENCE OF *CLOSTRIDIUM DIFFICILE* SPORES IN THE INTESTINAL EPITHELIUM AND ITS ROLE IN RECURRENT DISEASE

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*Clostridium difficile* is the most commonly reported nosocomial pathogen in developed and is some Latin American countries and is an urgent public health concern worldwide. One of the main clinical challenges of *C. difficile* infections (CDI) is the elevated rates of disease recurrence. During infection, *C. difficile* initiates a sporulation program that leads to the formation of spores that persist in the host. We have recently described how spores of an epidemically relevant strain binds to components of the intestinal mucosa *in vitro*. However, the mechanism of spore-persistence *in vivo* remains unclear. In this work, we demonstrate by transmission electron microscope and confocal microscopy how *C. difficile* spores interact with intestinal epithelial cells. *C. difficile* spore were found to be endocytosed by intestinal epithelial cells in an actin polymerization-dependent manner. *C. difficile* spores of various clinical isolates were able to internalize into intestinal epithelial cells, suggesting that this is a common feature for *C. difficile*. Using inhibitors combined with siRNA-based knockdown suggest that spore-entry requires part of the cellular endocytic machinery. We found that the internalization of *C. difficile* spores into intestinal epithelial cells is also dependent on molecular bridging molecules and integrin-receptors. Confocal microscopy of murine epithelium confirmed that entry of *C. difficile* spores also occurs *in vivo* in an illeal loop mouse model. Using a mouse model of recurrent infection, we observed that inhibition of spore-entry prevents recurrence of the disease. These results reveal a novel aspect of the pathogenesis of *C. difficile* by identifying a mechanism through which *C. difficile* spores are involved in the recurrence of the disease.

# MICROBIOTA OF MUCOSAL ASSOCIATED INVERIANT T CELL DEFICIENT MICE CONFER RESISTANTANCE AGAINST *CLOSTRIDIUM DIFFICILE* INFECTION

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*Clostridium difficile* (*Cd*) infection (CDI) typically occurs following antibiotic usage, which perturbs the gut microbiota leaving the host susceptible to *Cd* colonization. Development of mucosa-associated invariant T (MAIT) cells, a specialized subset of T cells that recognize intermediates of riboflavin biosynthesis only produced by microbes, is dependent on the host microbiome. MAIT cells are present at high proportions at mucosal sites and are beneficial in combatting pulmonary infections; however, their role in gut infection is unknown. To understand the role of MAIT cells in CDI, WT and $MR1^{-/-}$ (lacking MAIT cells) mice were treated with antibiotics and then inoculated with *Cd* spores. Stool was collected for 16S rRNA sequencing and plated to determine post-infection colonization levels. Surprisingly, $MR1^{-/-}$ mice showed no signs of disease or detectable levels of *Cd* colonization when infected with *Cd* 630 or *Cd* VPI 10463. 16S rRNA sequencing of fecal samples from each strain revealed stool from $MR1^{-/-}$ mice were significantly more rich (Chao1 index) than that from WT mice before and after antibiotic treatment. Fecal microbiota transplantation (FMT) was performed on antibiotic treated and germ-free mice to determine the role of the microbiota in this resistance phenotype. Susceptible (antibiotic treated or germ-free) WT mice given FMT from $MR1^{-/-}$ mice experienced dramatically lower colonization levels by day 7 and cleared detectable *Cd* by day 14, while WT mice given control FMT continued to exhibit high colonization levels. Our data suggest the $MR1^{-/-}$ gut microbiome is resistant to *Cd* colonization, and this resistance is transferrable via FMT.

*SXIII-5*

## IL-22 PREVENTS *CLOSTRIDIUM DIFFICILE* INFECTION VIA MODULATION OF MICROBIAL METABOLIC ACTIVITIES IN THE GUT

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*Clostridium difficile* infection (CDI) is a major cause of nosocomial infection in hospitalized patients. Mounting evidence demonstrates that the intestinal resident microbiota is essential for the protection against this pathogen. *C. difficile* does not expand and trigger infectious colitis in healthy individuals as long as their resident microbiota is intact. However, disruptions of the normal microbiota by antibiotics lead to the blooms of *C. difficile*, resulting in the development of CDI. Restoration of the normal microbial communities by transplantation with healthy microbiotas cures >95% of recurrent CDI. It is thought that the gut microbiota prevents enteric pathogen infections via activation of host antimicrobial immunity. However, the role of host immunity-mediated resistance conferred by the microbiota against *C. difficile* remains poorly understood. To address this question, we generated human microbiota-associated (HMA) mice by colonizing germ-free mice with fecal microbiota obtained from healthy human subjects. Using HMA mice, we found that the human-derived microbiota prevents the growth of *C. difficile* in the intestine. We have discovered the microbiota-conferred colonization resistance is dependent on the induction of interleukin-22 (IL-22) by innate immune cells. Based on the result of 16S rRNA sequencing and luminal metabolome analysis by CE-TOF/MS, we discovered that IL-22 signaling, activated by the resident microbiota, influences the re-structuring of the commensal microbiota, making it more resistant to CDI. A blockade of IL-22 signaling in HMA mice leads to perturbation of the gut microbial communities that affects the metabolism of dietary fibers in the gut. The alteration in the carbohydrate metabolism results in accumulation of intermediary metabolites of dietary fibers that fosters the abnormal growth of *C. difficile* in the gut. Thus, mucosal IL-22, induced by the human microbiota, prevents the colonization and proliferation of *Clostridium difficile* via modulation of microbial metabolic activities in the gut.

## OBESITY-ASSOCIATED GUT MICROBIOTA ENHANCES *CLOSTRIDIUM DIFFICILE* INFECTION IN MICE

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*Clostridium difficile* is the leading cause of nosocomial infections in the U.S. Obesity, which is a modern-day epidemic, increases the risk of acquiring *C. difficile* and is also associated with clinically severe *C. difficile* infection (CDI). However, the mechanism(s) that increase CDI susceptibility in obese individuals and lead to worse clinical disease remain unknown. Lack of animal models of obesity and CDI is one reason for this void. Thus, we established a novel animal model of CDI in obesity by coupling a mouse model of high fat diet (HFD)-induced obesity with CDI.

We show that compared to control (non-obese) mice, obese mice had longer duration of clinical disease (weight loss and diarrhea), inflammation and colonic tissue damage. Worse clinical disease in obese mice correlates with persistence of both *C. difficile* pathogen and toxins. During the early stages of infection, obese mice had slightly lower toxin levels compared to controls despite similar overall pathogen load, but the clearance of *C. difficile* bacteria and toxins was delayed in obese mice (detected in 80% of obese mice *vs* none in controls on day 15 of infection).

Host gut microbiota and metabolic environment can influence *C. difficile* lifecycle (sporulation and germination). Since we use purified spores for infection in our murine model and spore germination in the colon to toxin-producing vegetative cells is essential to establish disease, our findings suggest that obesity-associated colonic microenvironment could influence disease outcomes by regulating *C. difficile* lifecycle. In fact, transfer of microbiota from obese mice increased diarrhea and mortality in control mice after CDI. Overall our data indicate that obesity-associated changes in commensal bacteria could alter dynamics of the *C. difficile* life cycle and thus impact clinical disease. Current studies are focused on determining the composition of microbial and metabolite factors that alter *C. difficile* life cycle in obesity.


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***0800*** ***SESSION XIV:*** **ORAL PRESENTATION IV: *CLOSTRIDIOIDES (CLOSTRIDIUM DIFFICILE)* CLINICAL TRIALS I**

*SXIV-1*

## INSIGHTS FROM FIDAXOMICIN, BEZLOTOXUMAB AND SUROTOMYCIN CLINICAL TRIALS: LOOKING BEYOND THE PRIMARY ANALYSES

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A high degree of morbidity and mortality due to *C. difficile* infection (CDI) led the United States Centers for Disease Control and Prevention to designate *C. difficile* as an urgent threat. While vancomycin and metronidazole are effective in resolving initial infection in the majority of patients, return of symptoms in the days to weeks after the end of antibiotic treatment is common, with an increasing number of patients experiencing multiple recurrences due to relapse or reinfection. This has led scientists at academic centers, non-profit organizations and pharmaceutical companies to search for new therapies with the aim to reduce relapses and increase sustained cure. Three of these new therapies were developed by Merck or by companies acquired by Merck.

Fidaxomicin and bezlotoxumab successfully cleared regulatory hurdles and reached the market. The third therapy, surotomycin, while having favorable microbiologic and pharmacologic properties on par with fidaxomicin, and showing promising results in a Phase 2 trial, failed in Phase 3. Surotomycin is a cyclic lipopeptide antibiotic. It is rapidly cidal against *C. difficile* by disrupting cellular membrane activity in both logarithmic and stationary phases. The killing results in reduced toxin production and attenuates the immune response. Surotomycin has low oral bioavailability, allowing gastrointestinal tract concentrations to greatly exceed its MIC for *C. difficile*. The post antibiotic effect has been shown to be greater than 6 hours at relevant concentrations. It minimally disturbs normal gastrointestinal microbiota because of its lack of activity against Gram-negative anaerobes and facultative anaerobes. Phase 2 data suggested that surotomycin (250 mg 2X daily) is an effective CDI treatment, with statistically lower recurrence rates than 125 mg vancomycin 4X daily (17.2% vs 35.6%, p=0.035). So, what went wrong in Phase 3?

Differences in eligibility criteria, diagnostic methods, endpoint definitions, geographic location of study sites, and sample size for these three programs will be compared and contrasted. Data will be presented that demonstrate how study design variables can impact outcomes and how some of the design choices may have contributed to favorable or unfavorable outcomes for each program.

## SYN-004 (RIBAXAMASE) PREVENTS *CLOSTRIDIUM DIFFICILE* INFECTION AND ANTIMICROBIAL RESISTANCE

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SYN-004 (ribaxamase) is an orally-administered β-lactamase to be co-administered with IV β-lactam antibiotics. Ribaxamase remains in the intestinal lumen to degrade excess β-lactam antibiotics excreted in the bile. This is expected to protect the gut microbiome from disruption and thus prevent infections like *Clostridium difficile*, colonization by opportunistic pathogens and the emergence of antimicrobial resistance. SYN-004 was well tolerated in Phase 1 clinical studies and efficiently degraded ceftriaxone excreted into the human intestine in Phase 2 studies. SYN-004 did not alter the plasma pharmacokinetics of ceftriaxone as it was not systemically absorbed. A multinational, double blind, placebo controlled, efficacy study was conducted to determine whether SYN-004 could prevent *C. difficile* infection (CDI) with additional endpoints for colonization by opportunistic pathogens, changes in the balance of the gut microbiome and changes to the gut resistome. The study enrolled 412 patients who were admitted to the hospital for treatment of a lower respiratory tract infection. As treatment, the patients were expected to receive at least 5 days of IV ceftriaxone and were randomized 1:1 to receive either SYN-004 or placebo during ceftriaxone treatment and for a short time after. Patients could also receive oral macrolides as needed. Fecal samples were collected at three points for determination of bacterial colonization with specific pathogens and to examine changes to the gut microbiome and resistome. The patients were monitored for diarrhea (3 or more loose or watery stools in a 24 hour period) for 6 weeks during which CDI was defined as the presence of *C. difficile* toxin (as determined by the local laboratory). The study was powered at 80% for the reduction in CDI with 1-sided alpha = 0.05. The study met its primary endpoint with a 71% relative risk reduction in CDI ($p=0.045$) in the SYN-004 group vs. placebo and a statistically significant 44% relative risk reduction in new colonization by VRE ($p<0.001$). SYN-004 also reduced changes in the gut resistome. These data are consistent with SYN-004 protecting the gut microbiome thus preventing CDI, colonization by VRE and the emergence of antimicrobial resistance.

*SXIV-3*

# THE MICROBIOTA-BASED DRUG RBX2660 IS EFFICACIOUS AND SAFE IN PATIENTS WITH RECURRENT *CLOSTRIDIUM DIFFICILE* INFECTIONS: RESULTS FROM 2 CONTROLLED CLINICAL TRIALS

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**Purpose:** RBX2660 is a microbiota-based drug being developed to prevent recurrent *Clostridium difficile* infections (rCDI), an urgent public health threat. We compare results from two Phase 2 controlled trials to evaluate the safety and efficacy of RBX2660.

**Methods:** In a double-blinded multi-center Phase 2 clinical trial, patients were randomized to receive either 2 RBX2660 doses, 1 RBX2660 dose+1 placebo dose, or 2 placebo doses. This trial included an open-label phase in which patients who failed blinded treatment could receive ≤2 additional RBX2660 doses. In a separate multi-center open-label study, patients received ≤2 doses of RBX2660, and their outcomes were compared to patients in a matched historic standard-of-care antibiotic-treated control arm. Both studies enrolled patients with ≥2 prior rCDI or ≥2 prior CDI episodes requiring hospitalization. The primary efficacy endpoint was absence of CDI at 8 weeks from conclusion of treatment. Safety was assessed via patient diaries and clinical assessment, or by chart review for the control arm.

**Results:** 369 rCDI patients were evaluated across both trials. In the first trial, the efficacy among patients who received ≥1 blinded RBX2660 treatment was 66.7%(n=83) compared to 45.5% for placebo-treated patients(n=42; $p<0.05$). The efficacy among patients who received ≥1 RBX2660 in the open-label phase was 77.8%(n=54). In the second trial, efficacy among patients who received ≥1 RBX2660 treatment was 79.4%(n=136), compared to 51.8% in the Control group (n=110; $p<0.001$). Adverse events (AEs) within 8-weeks post-treatment were primarily gastrointestinal with no unanticipated AEs. There were no significant differences in the proportion of AEs or serious AEs among treatment groups within each trial. Furthermore, demographic variables of age, sex, or geographic location did not contribute to patient outcome.

**Conclusion:** Collectively, these controlled Phase 2 clinical trials demonstrate safety of RBX2660 in rCDI patients and effectiveness of RBX2660 in preventing rCDI when compared to placebo-treated and historical control groups.

This analysis was funded by Rebiotix Inc., Roseville, MN.

# MISOPROSTOL PROTECTS MICE AGAINST *CLOSTRIDIUM DIFFICILE* INFECTION AND ACCELERATES RECOVER OF GUT MICROBIAL DIVERSITY AFTER ANTIBIOTICS

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**Background**: Prostaglandin (PG)$E_2$ has beneficial effects on gastrointestinal epithelium. Inhibiting PGE2 production causes gut ulceration and inflammation. $PGE_2$ mimetics, such as misoprostol (MISO), have been used to prevent intestinal ulceration in patients taking PG synthesis inhibitors.

**Hypothesis**: MISO reduces the severity of *Clostridium difficile* infection (CDI) in antibiotic-treated mice and helps restore normal microbial diversity.

**Methods**: We used a mouse model of CDI using female C57/BL6 mice exposed to cefoperazone prior to gavage with spores of *C. difficile* (the NAP1/027 strain M7404 provided by Dr. Dena Lyras). Mice were exposed to MISO by intraperitoneal injection daily following infection. Survival and diarrhea severity were monitored. The impact of MISO on the recovery of the gut microbiota following exposure to antibiotics was measured using 16S rRNA gene sequencing in the absence of CDI.

**Results**: In a dose-dependent fashion, MISO improved survival and diarrhea severity following infection with *C. difficile*. Moreover, in antibiotic treated mice, MISO promoted recovery of the gut microbiota leading to increased alpha-diversity and more rapid restoration of perturbed members of the microbiota.

**Conclusions**: MISO appears to be protective in antibiotic-associated CDI and might restore colonization resistance following antibiotics and reduce the risk for CDI.

Funding: this work was supported by the Vanderbilt Digestive Disease Research Center and NIH Grants DK058404, TR001723, and AI121796.


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***0910*** ***SESSION XV:*** **THE "NON-*DIFFICILE*" IN CLOSTRIDIA: CURRENT CLINICAL & THERAPEUTC LANDSCAPE**

*SXV-1*

## ENTEROTOXIN-PRODUCING *CLOSTRIDIUM PERFRINGENS* TYPE F: MOLECULAR MECHANISMS AND CLINICAL SYNDROMES

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*Clostridium perfringens* type F strains producing the enterotoxin (CPE) are a major cause of human food poisoning (FP) and nonfoodborne gastrointestinal (GI) diseases (NFD) such as antibiotic-associated diarrhea. CPE is required for the intestinal pathogenicity of both FP and NFD strains. However, the clinical presentation of FP vs NFD differs, with FP usually being an acute illness, while NFD is often more chronic and severe. Variations between FP and NFD strains explain their transmission and clinical differences. CPE-producing FP strains form exceptionally resistant spores due to production of a variant small acid soluble protein, which facilitates their foodborne transmission by enhancing survival in improperly cooked/stored foods. In contrast, NFD strains typically produce the major NanI sialidase, which is not made by FP strains. Recent studies indicate that NanI sialidase contributes to intestinal colonization, likely by increasing adherence and growth. In vitro, NanI can promote CPE activity, suggesting it may increase the intestinal pathology of NFD strains.

Given the central role of CPE in both FP and NFD, there has also been effort to understand CPE action. Some CPE doses activate caspase-3 in cultured Caco-2 cells and recent studies determined that CPE also activates caspase-3 in mouse small intestine. However, kinetic analyses and inhibitor studies indicate that caspase-3 activation is not required for the development of CPE-induced intestinal damage or enterotoxemia (absorption of the toxin from the intestines to cause lethal effects). Instead, CPE-induced intestinal cell death and damage may involve a recently-discovered bystander killing effect or another unidentified cell death pathway. Last, the first CPE therapeutics may be on the horizon. Specifically, mepacrine has been shown to inhibit CPE cytotoxic activity in vitro and it also reduces CPE lethal activity in a mouse enterotoxemia model.

## BOTULINUM NEUROTOXIN-ENCODING PLASMIDS CAN BE CONGUGATIVELY TRANSFERRED TO DIVERSE CLOSTRIDIAL STRAIN

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*Clostridium botulinum* produces botulinum neurotoxin (BoNT), the most poisonous toxin known to humankind. Most Group I *Clostridium botulinum* strains harbor botulinum neurotoxin (*bont*) genes on their chromosome, while some carry these genes (including *bont/a*, *bont/b*, and *bont/f*) on large plasmids. Prior work in our laboratory demonstrated that Group I BoNT plasmids were mobilized to recipient *C. botulinum* strains that contained the Tn*916* transposon. In this study we have used conjugation protocols on agar media to study transfer of BoNT-encoding genes to various *Clostridium* species. We show that Tn*916* is nonessential for plasmid transfer. Relying on an auxotrophic donor phenotype and a plasmid-borne selectable marker, we observed the transfer of pCLJ, a 270 kb plasmid harboring two *bont* genes, from its host strain to various clostridia. The transfer frequency was greatest to other Group I *C. botulinum* strains, but the plasmid was also transferred into traditionally nontoxigenic species, namely *C. sporogenes* and *C. butyricum*. Expression and toxicity of BoNT/A4 was confirmed in transconjugants by immunoblot and mouse bioassay. These data indicate that conjugation within the genus *Clostridium* can occur across physiological Groups of *C. botulinum*, supporting horizontal gene transfer via *bont*-bearing plasmids. The transfer of plasmids possessing *bont* genes to resistant *Clostridium* spp. such as *C. sporogenes* could impact biological safety for animals and humans. These plasmids may play an environmental role in initiating death in vertebrates, leading to decomposition and nutrient recycling of animal biomass.

*SXV-3*

# THE IDENTIFICATION AND CHARACTERIZATION OF A NOVEL EXTRACELLULAR METALLOPROTEINASE PRODUCED BY *CLOSTRIDIUM SORDELLII*

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*Clostridium sordellii* is a significant pathogen for both animals and humans. Severe capillary leakage, toxic shock syndrome and an extreme increase in circulating leukocytes, termed the leukemoid reaction, are all hallmark features of this infection. *C. sordellii* produces several virulence factors including phospholipase, neuraminidase (NanS), and two large clostridial glucosylating toxins, TcsL and TscH. Recently, our group demonstrated that *C. sordellii* produced a novel metalloproteinase that cleaves human vascular cell adhesion molecule (VCAM)-1 *in vitro*, an receptor critical to hematopoietic precursor retention and leukocyte diapedesis. In the present study, we 1) established the predicted structure of Mcs1 using high-performance computing and high-dimensional data analytics; 2) characterized Mcs1's activity by means of a FRET-based *in vitro* protease assay, and 3) determined the *in vivo* role of Mcs1 utilizing a Δmcs1 mutant strain in our murine model of clostridial myonecrosis. Computational analytics successfully determined the protein structure of Mcs1 and confirmed the protein-protein interaction between Mcs1 and VCAM-1 bound molecules. Animal studies also demonstrated that Mcs1 plays an important role in the pathogenesis of *C. sordellii* infection. Specifically, mice infected with mutant strain outlasted those infected with wild-type organisms, and strains over producing Mcs1 produced rapidly fatal infections. Better understanding the role of Mcs1 in the the pathogenesis of *C. sordellii* infection may lead to the development of novel diagnostic tools or therapeutic strategies that could limit the morbidity and mortality associated with this deadly infection.

# CLOSTRIDIA, PRETERM NEONATES AND NECROTIZING ENTEROCOLITIS: NEW DATA

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**Background:** Necrotizing enterocolitis (NEC) is the most severe gastro-intestinal disease of preterm neonates (PN) that continues to account for substantial morbidity and mortality in neonatal intensive care units (NICUs). It is a multifactorial disease with an incompletely understood pathophysiology involving immaturity of the intestinal functions, enteral feeding and intestinal microbiota. If intestinal bacterial colonization plays a key role in NEC, data linking anaerobes and NEC physiopathology are scarse.

**Objectives:** Thanks to multi-centric studies, we performed a comparative study of clostridia strains isolated from NEC and control PN.

**Methods:** Fecal samples were obtained from PN recruited in two multicentric case-control studies (EPIFLORE (2011), ClosNEC (2015-2016)). Microbiota analysis was performed by 16S rRNA gene sequencing and bacterial culture. Stress responses, cellular cytotoxicity and inflammatory capabilities of 65 clostridia strains isolated from 29 NEC and 31 control PN fecal samples were compared. Statistics included quantitative (Wilcoxon-Mann and Whitney, $p < 0.05$ two-tailed) and categorical values (Fisher exact, $p < 0.05$) analysis. Phenotypic and inflammatory data were used as explanatory variables for NEC and control strains comparison (quadratic discriminant analysis).

**Results:** When strains characteristics were used as explanatory variables, a statistical discriminant analysis allowed the separation of NEC and control strains into separate groups. Strains isolated from NEC PN were characterized by a higher viability at 30°C ($p = 0.03$) and aero-tolerance ($p = 0.01$). NEC heat-treated bacteria induced higher Caco-2 cells IL-8 production ($p = 0.03$), suggesting pro-inflammatory activity. *In vitro*, bacteria, bacterial components, or fecal filtrates showed variable cytotoxic effects without specific association with NEC or control samples.

**Conclusion:** A systematic comparative analysis of clostridia strains characteristics support the existence of a specific bacterial signature associated with NEC that might be helpful as a biological marker.


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*SXVI-1*

## AN UPDATE ON THE MECHANISMS OF ACTION OF THE LARGE *C. DIFFICILE* TOXINS

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The pathogenesis of *C. difficile* is primarily mediated by the actions of two large clostridial glucosylating toxins, toxin A (TcdA) and toxin B (TcdB). The toxins act on the colonic epithelium and immune cells and induce a complex cascade of cellular events that result in fluid secretion, inflammation and tissue damage, which are the hallmark features of the disease. This talk will summarize our current understanding of the structure and mechanism of action of the *C. difficile* glucosylating toxins and the mechanisms by which antibodies can neutralize their function.

# HOST IMMUNE DEFENSE TO *C. DIFFICILE* INFECTION

Frisbee, A.L.;[1] Saleh, M.M.;[1] Simpson, M.E.; Donlan, A.N.; Cowardin, C.A.;[1,2] Buonomo, E.L.;[1,3] Petri, Jr., W.A.*[1]
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*Clostridium difficile* infection is the leading cause of hospital acquired antibiotic-associated diarrhea in North America. Deaths due to diarrheal diseases are the only infectious cause of death to be increasing in the United States, with the 5-fold since 1980 attributed in part to the emergence of the epidemic strain of *C. difficile*. These strains express the *C. difficile* Transferase toxin (CDT), in addition to Toxins A and B (TcdA and TcdB) and are more virulent and associated with higher mortality rates. We have recently identified a protective role for eosinophils against *C difficile* pathogenesis (Buonomo et al., 2016). We have also defined CDT's ability to increase host inflammation and suppress protective eosinophils through a TLR2 dependent mechanism (Cowardin et al., 2016). How CDT promotes virulence and eosinophil suppression via TLR2 is still under investigation. We employed a genome-wide microarray approach to reveal divergent transcriptional profiles between protected (TLR2-/-) and unprotected (WT) mice infected with either CDT expressing or CDT mutant strains of *C. difficile*. This work revealed novel host mediated TLR2-dependent inflammatory pathways to CDT. We provide an unbiased framework for understanding the host immune response to the binary toxin CDT produced by *C. difficile* and how TLR2 signaling enhances virulence.

Buonomo EL, Madan R, Pramoonjago P, Li L, Okusa MD, Petri WA Jr (2013) Role of IL-23 signaling in *Clostridium difficile* Colitis J Infect Dis 208:917-20.

Cowardin C, Kuehne S, Buonomo E, Marie C, Minton N, Petri WA. (2015) Inflammasome activation contributes to IL-23 production in response to *Clostridium difficile*. mBio 6:1-9.

Buonomo EL, Cowardin C, Wilson MG, Saleh MM, Pramoonjago P, Petri WA Jr. (2016) Microbiota regulated IL-25 protects from *Clostridium difficile* infection via eosinophils. Cell Reports 16:432-43.

Cowardin CA, Buonomo EL, Saleh MM, Wilson MG, Burgess SL, Kuehne SA, Schwan C, Eichhoff AM, Koch-Nolte F, Lyras D, Aktories K, Minton NP, Petri WA Jr. (2016). The binary toxin CDT enhances *Clostridium difficile* virulence by suppressing protective colonic eosinophilia. Nature Microbiology 1(8):16108.

*SXVI-3*

## STRUCTURE-FUNCTION ANALYSES OF THE PUTATIVE *CLOSTRIDIUM DIFFICILE* BILE SALT GERMINANT RECEPTOR, CspC

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*Clostridioides difficile*, also known as *Clostridium difficile*, is a leading cause of antibiotic-associated diarrhea worldwide. In order for this obligate anaerobe to initiate infection, its spore form must germinate in the gut of susceptible hosts. Upon sensing bile salts in the gut, *C. difficile* spores will initiate a signaling cascade that will allow them to exit dormancy and eventually outgrow to form toxin-producing vegetative cells. A genetic screen previously identified the CspC pseudoprotease as the germinant receptor for bile salts. CspC is a member of the Csp family of subtilisin-like serine proteases that either directly or indirectly activates the CspB protease, a related member of the subtilisin-like serine protease family. To gain insight into the mechanisms controlling CspC function, we have solved the 1.6 Å structure of CspC. The overall structure closely aligns with the related protease CspB, with a jellyroll domain interrupting the core subtilase domain, and an N-terminal prodomain closely associated with the protease domain. However, several structural features distinguish the CspC pseudoprotease from previously studied subtilisin-like serine proteases. We will discuss these features along with structure-guided mutational analyses in *C. difficile* that provide novel insight into the mechanism by which CspC activates spore germination.

# ALTERATIONS IN THE GUT METABOLOME AND *CLOSTRIDIUM DIFFICILE* TRANSCRIPTOME IN A MOUSE MODEL OF INFECTION

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*Clostridium difficile* is a spore-forming bacterial pathogen that causes a range of clinical disease from mild to moderate diarrhea, to pseudomembranous colitis, and death. Typically, *C. difficile* infections (CDIs) occur after antibiotic treatment, which alters the gut microbiota and decreases colonization resistance against *C. difficile*, however the mechanisms driving colonization resistance are not well understood. One possible mechanism is nutrient inaccessibility or competition in the complex ecosystem of the gastrointestinal tract. We explored this hypothesis using an untargeted metabolomics approach (GC- and LC/MS-based) to define the metabolites present in the ceca of antibiotic-treated, *C. difficile* infected mice at the following time points: 0, 12, 24, and 30 hours. Significant changes in amino acid and carbohydrate metabolism were observed throughout CDI. In particular, Random Forest analysis identified increased 5-aminovalerate as an important contributor to differences between time points. 5-aminovalerate is produced via proline fermentation, and as *C. difficile* is auxotrophic for six amino acids, including proline, the increase in 5-aminovalerate suggests that early acquisition of amino acids is a priority within the gut environment in this model. RNA-Seq on paired cecal content identified >500 differentially expressed genes, suggesting that *C. difficile* alters its gene expression to accommodate changes in nutrient sources over time. In addition to genes whose products are involved in carbohydrate and amino acid uptake and fermentation, we identified four and eight proteases or peptidases as being increased in expression at 24 and 30 hours, respectively. Understanding the relationship between nutrient preference, acquisition, utilization, and *C. difficile* colonization will lead to the development of targeted bacterial therapeutics able to outcompete *C. difficile in vivo*.


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*SXVII-1*

## NEW IDSA/SHEA GUIDELINES FOR CDI DIAGNOSIS AND TREATMENT

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The 2017 IDSA/SHEA clinical practice guidelines for CDI now include recommendations for children and major changes in the diagnosis and treatment of adults with CDI. The guidelines also reflect the adoption of nucleic acid amplification tests (NAAT) such as PCR over toxin testing in the majority of US clinical laboratories since 2010. The decision to use NAAT testing over an algorithm that includes toxin testing should be based on pre-agreed institutional criteria for patient stool submission that excludes specimens from patients without substantial diarrhea or who are taking laxatives. Routine testing is only recommended for children over 2 years of age who have prolonged or worsening diarrhea and risk factors for CDI or relevant exposures.

Based on high quality evidence from 5 randomized, controlled comparative trials (RCTs) with vancomycin, metronidazole is no longer recommended as first line treatment for adult patients with initial episode of CDI regardless of disease severity. High quality evidence also guided recommendations for fidaxomicin as another option for treatment of initial CDI episodes. Vancomycin, fidaxomicin, or vancomycin taper and pulse regimen are recommended for a first recurrent episode depending on the treatment for the initial CDI episode, but these are weak recommendations based on much lower quality of evidence. For patients with multiple recurrences who have failed appropriate antibiotic treatments (i.e., for at least 2 recurrent episodes) fecal microbial transplantation (FMT) is recommended, but an FDA-approved formulation is not yet available and appropriate donor screening and obtaining informed consent from the recipient is required. High quality evidence for treatment of pediatric patients is not yet available and metronidazole is recommended for treatment of initial CDI episodes in children, but only in patients with non-severe disease. There was insufficient data to recommend extending the duration of CDI treatment when the precipitating antibiotic is not stopped or restarting empiric CDI treatment in patients who have recovered from CDI but who are re-administered systemic antibiotics. These guidelines will be updated when new data and experience with new agents become available.

## FECAL MICROBIOTA TRANSPLANT AND DERIVATIVES FOR CDI

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*C. difficile* infection (CDI) is the commonest cause of nosocomial diarrhea and also an important cause of diarrhea in the community. There has been a change in epidemiology of this infection and this increased incidence and severity of CDI and associated increased morbidity and mortality can be attributed in part to emergence of a hypervirulent strain along with increased use of antibiotics and proton pump inhibitors. Additionally, there are several challenges in the management of primary CDI with antibiotics which leads to increased recurrences with rates up to 60% after 3 or more CDI episodes. Recent treatment guidelines (IDSA / SHEA 2017) for CDI recommend decreasing the use of metronidazole and using vancomycin or fidaxomicin.

The pathophysiology of recurrent CDI involves the disruption of normal gut flora and an increased predisposition to CDI secondary to the use of antibiotics including antibiotics used to treat CDI. These latest guidelines also recommend the use for fecal microbiota transplantation (FMT) for management of recurrent CDI. FMT is emerging as a safe and effective treatment for the management of recurrent, and possibly refractory, CDI by restoring gut microbial diversity with high cure rates. Initial randomized clinical trials of traditional FMT for recurrent CDI have shown cure rates in the 85-90% range. The currently available FMT for recurrent CDI is heterogeneous and there is a need to standardize donor screening and transplant methodologies. Emerging treatments for microbiota restoration for CDI include enema and capsule based therapies. There are several products that are being commercially developed and are undergoing phase II and phase III clinical trials. This presentation will summarize the existing literature on FMT for recurrent CDI and highlight ongoing clinical trials.

*SXVII-3*

## UPDATE ON NEWER MANAGEMENT OF *CLOSTRIDIUM DIFFICILE* INFECTION (CDI)

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Newer approaches to CDI management that will be discussed include the following:

New antibiotics with very narrow spectrum that target *C. difficile* while sparing the microbiome are under development. Ridinilazole appears very promising based on phase 2 results, but surotomycin and cadazolid have failed in blinded trials vs. vancomycin.

Extension of the dosing period of approved antibiotics using pulse dosing as has been done with fidaxomicin in the EXTEND open label trial and in observational studies of extended pulsed dose fidaxomicin and vancomycin for recurrent CDI.

Monoclonal antibody targeted at *C. difficile* toxin B; bezlotoxumab.

*C. difficile* vaccines are currently in phase 2 and 3 trials but one vaccine development program has been discontinued.

Fecal microbiome transplants.

Biotherapeutics which include use of non-toxigenic *C. difficile* and subsets of microbiota organisms from stool such as SER-109 and combinations of spore forming organisms produced under good manufacturing practice.

Antibiotic inhibitors or binders to reduce antibiotic concentration in the gut to preserve the microbiota.

These approaches may be used either for treatment or for primary or secondary prevention of CDI and raise the possibility of significantly reducing the incidence of CDI if successful.

***1530 SESSION XVIII: ORAL PRESENTATIONS V: CLOSTRIDIUM DIFFICILE—CLINICAL TRIALS II***

*SXVIII-1*

## ORAL BETA-LACTAMASE THERAPY PROTECTS THE GUT MICROBIOME FROM IV AND ORAL ANTIBIOTICS AND MITIGATES PROPAGATION OF ANTIBIOTIC RESISTANCE

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Antibiotics can disrupt the gut microbiome leading to overgrowth of pathogenic organisms and propagation of antibiotic resistance. SYN-004 (ribaxamase) is a clinical-stage, oral beta-lactamase intended to degrade certain IV beta-lactam antibiotics in the GI tract to preserve the gut microbiome. Ribaxamase was evaluated in a phase 2b study that met its primary endpoint of significantly reducing *C. difficile* infection in patients treated with IV ceftriaxone. In pigs, ribaxamase protected the gut microbiome from IV ceftriaxone and reduced emergence of antibiotic resistance. To expand microbiome protection to include oral antibiotics, new ribaxamase formulations, named SYN-007, were engineered for release in the lower small intestine distal to the site of antibiotic absorption. Three SYN-007 formulations (10 mg, PO, TID) were evaluated in dogs treated with oral amoxicillin (80 mg/kg, PO, TID) for 5 days. Systemic amoxicillin levels were not significantly different +/- SYN-007 after first and last amoxicillin doses for one formulation. Fecal DNA metagenomic analyses demonstrated gut microbiome alterations and emergence of antibiotic resistance genes in dogs that received amoxicillin alone, while microbiota changes and antibiotic resistance were diminished with SYN-007. Ribaxamase degrades penicillins and cephalosporins but not carbapenems. To broaden this prophylactic approach to all classes of beta-lactam antibiotics, SYN-006, a metallo-beta-lactamase derived from *B. cereus,* was formulated for oral delivery. Pigs received ertapenem (30 mg/kg, IV, SID) +/- SYN-006 (1 mg/kg, PO, QID) for 4 days. Serum ertapenem levels were not significantly different +/- SYN-006. Microbiome analyses are in progress. Antibiotic inactivation represents a new treatment paradigm for preservation of the gut microbiome and reduction of antibiotic resistance. Ribaxamase, SYN-007, and SYN-006 have the potential to protect the commensal gut microbiota from antibiotic damage and to mitigate emergence and spread of antibiotic resistance.

# DEVELOPING MICROBIOME REHABILITATION BIOMARKERS FOR *CLOSTRIDIUM DIFFICILE* INFECTIONS: CONTINUED EVALUATION OF A PROTOTYPE MICROBIOME HEALTH INDEX

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**Purpose:** To explore a potential biomarker of microbiome restoration in patients with recurrent *Clostridium difficile* infections (rCDI), we evaluated unidimensional Microbiome Health Indices (MHI) from two Phase 2 clinical trials of RBX2660, a standardized microbiota-based therapeutic with demonstrated clinical efficacy for preventing rCDI.

**Methods:** MHIs were calculated from sequencing data from patient fecal samples and RBX2660 product samples from a randomized, blinded, placebo-controlled Phase 2B trial and from an open-label Phase 2 trial of RBX2660 to prevent rCDI. Data from the Phase 2B trial were based on 16S sequencing and data from the open-label trial were based on shallow shotgun sequencing. MHI values from the two trials were compared and pooled. Receiver operator characteristic (ROC) analysis was used to define an MHI cut-point for distinguishing rCDI subjects prior to treatment (baseline) from the RBX2660 drug product. Post-treatment MHIs from patients were assessed longitudinally and by outcome.

**Results:** Baseline and RBX2660 MHI values were not significantly different between the two trials, despite data derivation from different sequencing methods ($p>.05$). ROC analysis indicated that the pooled baseline samples could be distinguished from the pooled RBX2660 profile with a maximum likelihood ratio of 121 (AUC=0.99, sensitivity=0.96, specificity=0.99, cutpoint=8.2). Among patients who responded to treatment, MHIs were significantly higher 7±4 days after treatment ($p<.001$) with 58% of responders having an MHI>8.2. Among patients who failed treatment, only 21% were above the MHI=8.2 cutpoint. More importantly, MHI of successes could be distinguished from failures at 7±4 days post-treatment ($p=.003$, Wilcoxon test).

**Conclusion:** MHI values are consistent across two trials using two different sequencing methods, suggesting generalized utility. MHI can effectively distinguish patients with dysbiosis from healthier patients and can differentiate successes from failures post-treatment. These results generate prospectively evaluable hypotheses for future clinical trials and emphasize the value of a unidimensional MHI.

This analysis was funded by Rebiotix Inc., Roseville, MN.

*SXVIII-3*

## GASTROINTESTINAL TRACT MICROBIOME DYNAMICS FOLLOWING TREATMENT WITH SER-109, AN INVESTIGATIONAL ORAL MICROBIOME THERAPEUTIC TO REDUCE THE RECURRENCE OF *CLOSTRIDIUM DIFFICILE* INFECTION (CDI)

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CDI recurrence occurs within a few weeks after treatment due to antibiotic-induced dysbiosis. SER-109, an investigational, first-in-class microbiome drug, was designed to sustain a clinical response through microbiome restoration. In an open-label Phase 1b (Ph1b) trial of SER-109 for prevention of recurrent CDI, 26 of 30 subjects did not recur following treatment. In a Phase 2 (Ph2) double-blind controlled trial of SER-109 (n=59) vs placebo (n=30), no significant difference was observed in the proportions of subjects with recurrence (44.1% vs 53.3%, respectively). We contrast gut microbiome changes among subjects in both trials to understand differences in clinical outcomes observed 8-weeks after dosing, and the impact of treatment on carriage of antibiotic resistance associated bacteria.

Using 16Sv4 and whole metagenomic shotgun sequencing, we observed significantly greater richness of commensal spore-former species in subjects treated with SER-109 compared to PBO at weeks 1 and 4 post-treatment ($p=0.008$, $p=0.044$), consistent with drug engraftment. The number of spore-forming species at 1 week was significantly greater in non-recurrent subjects vs recurrent subjects with a positive EIA toxin test ($p=0.011$). We identified 10 spore-former species that were significantly more prevalent in both SER-109 and non-recurrent subjects. In comparison to Ph1 subjects, SER-109 engraftment was significantly reduced and delayed in Ph2 subjects. Moreover, Ph1 subjects who received higher doses of SER-109 than that used in Ph2 had increased levels of engraftment.

In patients with recurrent CDI and dysbiosis, a focused spore-based therapeutic approach leads to engraftment of SER-109 strains. In addition, microbiome signatures of engraftment were associated with a favorable clinical outcome and a reduction of antibiotic resistance associated genes. Although SER-109 was biologically active, a higher dose may improve the rate and degree of microbiome repair.

## RIDINILAZOLE (RDZ) FOR *CLOSTRIDIUM DIFFICILE* INFECTION (CDI)

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CDI is the most common hospital acquired infection in the USA with recurrent CDI a particular concern. RDZ is a novel antimicrobial for CDI with a highly targeted spectrum of activity expected to reduce collateral damage to the gut microbiota during therapy. Here we report data from the CoDIFy proof-of-concept Phase 2 clinical trial.
This multi-centre, double-blind, randomized, active-controlled study randomized 100 patients 1:1 to 10 days RDZ 200 mg BID or vancomycin (VAN) 125 mg QID treatment. Clinical response was assessed 2 days after end of therapy (EOT). The primary endpoint was non-inferiority on sustained clinical response (SCR), defined as clinical response at EOT with an absence of recurrent disease for the next 30 days. The primary analysis population was the modified intent-to-treat (MITT) which included all randomized subjects with a diagnosis confirmed by presence of free toxin in stool.

The study exceeded its primary endpoint, with RDZ shown to be superior on SCR to VAN with rates of 66.7% and 42.4% respectively (difference in treatment proportions 21.1%; 90% CI 3.1, 39.1). Rates of clinical cure at EOT were 77.8% and 69.7% for RDZ and VAN, respectively (difference in treatment proportions 8.3%; 90% CI -9.3, 25.8). When SCR for the MITT was analysed across subgroups at high risk of recurrence, RDZ was favoured over VAN with estimated improvements (90% CI) for patients >75 years of age of 42.7% (9.7, 75.7), for severe disease of 15.9% (-29.8, 61.6) and for prior episodes of CDI of 19.9% (-22.8, 62.5). Analysis of the microbiome of subjects showed that the alpha diversity decreased following administration of both antibiotics, although the change was significantly less with ridinilazole ($p>0.0001$.) Beta diversity analysis also showed a significantly larger weighted Unifrac distance from baseline to end-of-therapy with vancomycin. When the effects on specific taxa were examined, the markedly narrower impact of ridinilazole was clear. At end-of-therapy, significant reductions in the percent relative abundance following ridinilazole were only found within the phylum Firmicutes.

RDZ has been shown in a randomsied clinical trial to be highly effective at reducing recurrent CDI which is likely due to its microbiome sparing characteristics. Further clinical development in Phase 3 studies is warranted.

*SXVIII-5*

## HOW ABOUT PROGRESS TOWARDS THE DEVELOPMENT OF A PROPHYLACTIC *CLOSTRIDIUM DIFFICILE* VACCINE

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*Clostridium difficile* (*C difficile*) diarrhea is a cause of antibiotic-associated nosocomial and community acquired infection. Risk factors for disease include antibiotic use, interactions with healthcare systems and age, with risk factors starting to increase for the over 50 year old age group. To date, there is no vaccine available to prevent *C. difficile* infection (CDI). The Pfizer *C.difficile* vaccine (PF-06425090) initiated a Phase 3 clinical efficacy study which is known as CLOVER (*Clostridium difficile* Vaccine Efficacy Trial) in 2017 which is currently ongoing. The vaccine is comprised of genetically and chemically detoxified, purified toxins (toxoids) A and B formulated with Al(OH)3. The vaccine has been shown to be well tolerated in early phase clinical studies and induces neutralizing antibody responses that can neutralize diverse *C. difficile* toxins. Results from these early studies will be discussed.

*1315* ***STUDENT POSTER SESSION:*** **STUDENT COMPETITION**



Posters will be presented in Student Poster Competition
Tuesday, July 10 1200-1320.

*SP-1*

## MUCUS-DEGRADING BACTERIA MODULATE MUCOSAL ADHERENCE OF GENOTOXIC BACTERIA FOR PROMOTING COLON TUMORIGENESIS

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Colon tumorigenesis in azoxymethane (AOM)-treated wild type mice is remarkably induced by co-colonization of enterotoxigenic *Bacteroides fragilis* (ETBF) and adherent-invasive *Escherichia coli* (*E. coli*) NC101. Synergistic mechanisms of ETBF and *E. coli* NC101 in causing colon tumorigenesis requires *Bacteroides fragilis* toxin (BFT), BFT-induced chronic inflammation, and genotoxin Colibactin encoded by *E. coli* NC101 *pks* (polyketide synthase) pathogenicity island. Mechanistically, ETBF increases the contact of *pks+ E. coli* with colonic epithelial cells by enhancing mucosal adherence of *pks+ E. coli*, which is prerequisite for Colibactin to cause double-strand DNA breaks. We sought to define the molecular mechanisms by which ETBF promotes *E. coli* mucosal adherence. According to Freter's nutrient-niche theory, competition for nutrients is the most important factor that influences pathogenic bacteria colonization. We, therefore, hypothesize that ETBF promotes *pks+ E. coli* mucosal localization and growth by supplying certain nutrients from mucus layer. Using bacterial cultivation with porcine gastric mucin, we found that *Bacteroides fragilis* species have a general feature to utilize mucin as the sole carbon source, whereas *pks+ E. coli* alone is not capable to grow in mucin medium. *pks+ E. coli* growth was promoted in mucin medium conditioned by ETBF or *Akkermansia muciniphila* (*A. muc*, a known mucin degrader). Conditioned mucin medium analyzed by SDS-PAGE gel electrophoresis and Coomassie staining suggested that polypeptides derived from ETBF-degraded porcine gastric mucin exhibited a different pattern as compared to that from *A. muc*-degraded mucin. Seeing ETBF and *A. muc, as mucin degraders,* may support *pks+ E. coli* growth in vitro through different mechanisms, we will further our research to explore the nutrients provided by ETBF-degraded colonic mucin in comparison with *A. muc*. These results suggest that, distinct from commensal mucin degrader *A. muc*, ETBF may employ unique mucin-degrading mechanisms to promote mucosal adherence of *pks+ E. coli*, which is highly likely to impact colon tumorigenesis.

## IDENTIFICATION OF A NOVEL LAMININ LIGAND IN *PREVOTELLA NIGRESCENS*

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*Prevotella spp*. is a genus of Gram negative, strict anaerobic bacteria associated with opportunistic infection in the mouth, female genital, respiratory and gastrointestinal tract. During infection, adhesion to host tissue is essential for establishment and persistence of bacteria. Here we investigated interaction between ATCC strains of oral Prevotella species, *P. intermedia* (ATCC49046), *P. melaninogenica* (ATCC25845) and *P. nigrescens* (ATCC33563) with laminina. Laminin is one of the main components of basement membrane, which separates body surfaces such as epithelium and endothelium from connective tissue. Initially, to evaluate binding, we allowed increasing concentrations of bacteria ($5.10^7$ and $10^7$ UFC/mL) to interact with mouse laminin immobilized in glass coverslips. Adhered cells were stained with an unspecific dye and viewed with fluorescence microscopy. We captured 10 random images of each coverslip [Field of vision (FV) = 0,049 mm²] and quantified adherence with Image J software. *P. melaninogenica* adherence was 599,5 UFC/FV and 141 UFC/FV, adhesion to negative control (1% BSA) was 38 UFC/FV. *P. nigrescens* adherence was 316,6 UFC/FV and 155,4 UFC/FV negative control was 98,1 UFC/FV. We did not detect any adherence of *P. intermedia.* To identify laminin ligands in *Prevotella*, outer membrane extracts were purified from *P. nigrescens* and ran through an NHS-activated sepharose affinity columns containing immobilized laminin. Unbound material was washed from the column and the laminin-bound proteins remaining were eluted with Glycine buffer. Eluted fractions containing potential ligands were analyzed by SDS-PAGE. Protein bands were excised from the gel and analyzed by mass spectrometry. We identified a 1481 amino acid putative adhesin protein (Accession number WP_040557291.1) which function as a laminin ligand in *P. nigrescens.* Blast search confirmed the presence of genes coding this protein in the genome of *P. nigrescens* ATCC33563 as well as several other *P. nigrescens*. To our knowledge, this is the first time that binding activity is described for this putative adhesin. Understanding molecular mechanisms involved in adhesion may help us design new strategies to prevent periodontitis and biofilm formation in gingival sulcus.

*SP-3*

## USING CRISPR-CAS9-MEDIATED GENOME EDITING TO GENERATE *C. DIFFICILE* MUTANTS DEFECTIVE IN SELENOPROTEINS SYNTHESIS

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*Clostridioides difficile* is a significant concern as an opportunistic pathogen and a major cause of antibiotic-associated diarrhea. Much of our understanding of *C. difficile* physiology has come in the last few years and coincided with the development of genetic tools for this organism. Though the current genetic tools for *C. difficile* have been beneficial, they each have negative aspects (*i.e.*, time-consuming, multi-step processes, inefficient and polar/off-target effects). The clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) system has gained attention as a genetic tool in several organisms, and this system was recently shown to work efficiently in other Clostridia. To apply such a system to *C. difficile* for genetic modification, we created a CRISPR-Cas9 plasmid which contains all of the components necessary for the system to function. We targeted and made a clean deletion of *pyrE*, which encodes orotate phosphoribosyltransferase and can be used easily as a counter-selection, in *C. difficile* R20291. Using this system we achieved an efficiency of ~ 50% with no off-target effects. To investigate the role of selenoproteins in *C. difficile* Stickland metabolism, a primary source of energy in a small number of anaerobic bacteria, we applied our genetic tool to make a clean deletion of *selD*, a gene which encodes a selenophosphate synthetase, the first step of selenium incorporation into proteins. We found that *selD* has an important role in the growth of *C. difficile* where a *selD* mutant has a growth defect in protein-rich medium. This newly-developed *C. difficile* genetic system builds upon and improves upon the available genetic systems. Because this system does not rely upon segregationally unstable plasmids or pre-existing/generated *pyrE* mutations, this system should increase the rate with which mutations can be made in *C. difficile*.

## NANR REGULATION OF SPORULATION AND CPE PRODUCTION IN *CLOSTRIDIUM PREFRINGENS*

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*C. perfringens* type A strains producing the enterotoxin (CPE) are a major cause of gastrointestinal (GI) infections. These strains are responsible for the second most prevalent bacterial food-borne illness, as well as 5-20% cases of antibiotic-associated diarrhea. Virulence of this bacterium can be primarily ascribed to CPE, which is synthesized during sporulation. In addition, some strains carry a *nan* operon that facilitates *C. perfringens* metabolism of sialic acid liberated from surrounding macromolecules by the exosialidase NanI. In *C. perfringens* strain F4969, both *nan* operon expression and NanI activity have been previously demonstrated to be upregulated by the transcriptional regulator NanR; the current study has further investigated the influence of NanR on F4969 sporulation and CPE production. An isogenic F4969 *nanR* null mutant displayed limited sporulation and CPE production but increased NanI production, indicating that NanR positively regulates sporulation and CPE synthesis but represses NanI production. Our previous study showed that decreasing NanI production promotes both spore and CPE formation, as the F4969 *nanI* null mutant strain produced more spores and CPE than did wild-type F4969. To establish that NanR directly affects sporulation and CPE synthesis, rather than indirectly through regulation of NanI, a *nanI-nanR* double null mutant was utilized. This strain mirrored the outcome of the *nanR* null mutant strain, with reduced sporulation and CPE. Collectively, these results imply that *nanR* is a crucial gene for the positive regulation of sporulation and CPE formation in *C. perfringens*.

*SP-5*

## SOIL MAY BE AN IMPORTANT RESERVOIR FOR *CLOSTRIDIOIDES DIFFICILE* DISEASE IN FLAGSTAFF, ARIZONA

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*Clostridioides* (formerly *Clostridium*) *difficile* has been considered a nosocomial infection, but recent research suggests that community-acquired infections are also important. However, little is known about bacteria sourced outside the clinical environment. Although surveyed in other countries, the prevalence of *C. difficile* in the outdoor environment in the United States is largely unknown. Our goal was to characterize *C. difficile* in the environment in Flagstaff, AZ, a relatively small city (population ~72,000) that is serviced by a single hospital, in an effort to determine the role that environmental *C. difficile* may play in disease. In this study, we collected 53 soil samples from Flagstaff. Samples were processed per published methods in an anaerobic chamber using media specific for culturing *C. difficile*. Genomic DNA was extracted from culture and a multiplex real-time PCR assay was used to determine if cultures were positive for *C. difficile* and to determine the presence/absence of the *tcdB* gene, an indicator of toxin production. PCR results revealed that 36 of the 53 soil samples (68%) were positive for *C. difficile*, and 24 of 53 (45%) were positive for the *tcdB* gene. Additionally, three pure cultures of *C. difficile* were obtained from two soil samples. Genomic DNA from pure isolates was whole genome sequenced on an Illumina MiSeq platform and compared to other *C. difficile* obtained from human and canine fecal samples from Flagstaff, AZ. One of the soil isolates was genetically similar to sequences from both a human and a canine fecal sample. The remaining two soil isolates were genetically distinct from the other 271 whole genome sequences in our database. These data support that *C. difficile* is ubiquitous in the environment around Flagstaff and demonstrate that toxin-producing strains prevail outside of a host. They also allude to the diversity of *C. difficile* that exists within soil. Finally, genetic similarity between a soil and a clinical sample indicates that soils are possible reservoirs for infection in Flagstaff, AZ.

# A POTENTIAL SOLUTION TO A POOPY PROBLEM: BILE SALT ANALOGS AS PROPHYLACTICS AGAINST *CLOSTRIDIUM DIFFICILE* INFECTION

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*Clostridium difficile* infection (CDI) is a major cause of antibiotic-associated diarrhea. In 2011, over 500,000 patients were diagnosed with CDI in the United States and over 29,000 people died of CDI-related complications. Cost burden to the healthcare system can reach up to $3.2 billion annually. With rises in both hospital- and community-acquired CDI incidences due to the emergence of hypervirulent strains, CDI recurrences can reach up to 25%. Thus, standard treatments are rendered less effective, making new methods of prevention more critical.

CDI is caused by *Clostridium difficile*, a bacterium that can form tough and dormant structures called spores. The spores' dormant nature allows them to survive in the gastrointestinal tract of susceptible patients without showing any signs of infection. When the spores are under stress, they can germinate into toxin-producing cells that cause symptomatic infection. Since spore germination is a necessary step for CDI establishment, methods that target this process could serve for infection prophylaxis.

*Clostridium difficile* spore germination is promoted by the bile salt taurocholate. Previously, CamSA, a synthetic bile salt analog of taurocholate, was found to be a more potent germination inhibitor than CDCA when tested against strain 630. In the present study, a new analog, SKS-VI-07C, was found to be a stronger germination inhibitor than CamSA against strain 630 spores. SKS-VI-07C was also shown to be an effective anti-germinant against seven other strains representing different *C. difficile* ribotypes, including hypervirulent strain R20291. Germination inhibition assays showed that SKS-VI-07C inhibited spore germination in each of the *C. difficile* strains tested at concentration below 50 μM. Furthermore, mice challenged with each of the *C. difficile* strains had significantly reduced CDI signs, delayed symptom onset, or were completely protected from CDI. From these explorations, bile salt analogs may have the potential to serve as CDI prophylactic treatments in antibiotic-treated patients.

*SP-7*

## ANALYSIS OF *CLOSTRIDIUM DIFFICILE* SPORE GERMINATION IN FLUID FROM THE SMALL INTESTINAL TRACT OF HEALTHY ADULTS

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Following perturbations in the gut microbiota, *Clostridium difficile* invades the gut and causes diarrhea and colitis. *C. difficile* spores are thought to germinate in the small intestine in the presence of bile salts and co-germinants. The primary objective of this study was to identify characteristics of the human small intestine that modulate *C. difficile* spore germination.

Using a multilumen tube introduced into the gastrointestinal tract via the oral cavity, intestinal fluid was sampled from the duodenum, jejunum, and ileum of healthy, fasted individuals. Fluid was aseptically collected and stored for 16S microbial community analysis in consideration of low sample biomass. Using *C. difficile* strain R20291, spore germination was tested through ex vivo assays in fluid buffered at pH 7. Statistical methods, such as linear regression and LEfSe, were used to analyze the data.

This study included 19 individuals (Sex: 13 M, 6 F; Age: 33.6 ±9.4; BMI: 26.7 ±6.1). Fluid pH was lowest in the duodenum (4.7 ±1.9) and highest in the mid jejunum (6.42 ±0.26). Ex vivo germination assays at pH 7 demonstrated that fluid from all sections of the small intestine support *C. difficile* germination. However, germination efficiency was lower in duodenal and distal jejunal fluid. Linear regression showed that location is a significant predictor of germination ($p = 0.047$). 16S analysis indicated the presence of *Clostridium* cluster XIVa taxa, including *Clostridium scindens* which is known to metabolize bile salts and partially protect gnotobiotic mice from *C. difficile*. LEfSe revealed a significant correlation between *Stomatobaculum*, a Lachnospiraceae, and low germination efficiency ($p = 0.018$).

Although germination occured in fluid from all regions of the healthy human small intestine, a gradient exists where germination efficiency was lower in distal small intestinal fluid compared to proximal regions. These findings also suggest that the certain bacterial species are correlated with a germination phenotype. Future research on the role of specific bile salts and microbes may lead to new probiotic treatment options.

# ASYMPTOMATIC CANINE PETS MAY SERVE AS A SOURCE OF COMMUNITY ACQUIRED *CLOSTRIDIUM DIFFICILE* INFECTION IN HUMANS

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We established the prevalence and diversity of *Clostridium difficile* in domestic canines from Flagstaff, Arizona, and compared our findings to strains that cause human disease to explore the hypothesis that canines are asymptomatic carriers of toxigenic strains. Hospital acquisition of *C. difficile* is well documented, yet multiple studies implicate animals as possible sources of community acquired *C. difficile* infections (CDIs) in humans. To explore the potential role of dogs in human CDIs we systematically collected canine fecal samples (*n*=216) to investigate three important questions: 1) What is the prevalence and diversity of *C. difficile* in this pet population, 2) do *C. difficile* isolates collected from canines genetically overlap with isolates that can cause human disease, and 3) do *C. difficile* positive fecal samples display signatures of gastrointestinal disease (i.e. microbial dysbiosis)? We used a qPCR assay to screen all fecal samples for the presence of *C. difficile*. Culturing was then attempted on positive samples using an anaerobic growth chamber. All cultured isolates were analyzed with two sequence-based approaches: 1) multilocus sequence typing to broadly evaluate genetic diversity, and 2) whole-genome sequencing to assess finer-scale patterns. Finally, we used 16S rRNA microbial community analysis to compare stool samples from *C. difficile* positive and negative dogs to identify disruptions to the gut microbiome. We detected *C. difficile* in 17% of the canine samples, with 10% carrying toxigenic strains that cause human disease. Our sequencing analyses revealed similar genotypes and fine-scale diversity patterns in dogs and humans, which may suggest the potential for *C. difficile* transmission between them. Furthermore, the presence of this pathogen was not associated with major changes in the microbial community, which implies *C. difficile* tolerance in the canine gut. Together, these findings suggest that canine pets carry disease causing strains of *C. difficile* asymptomatically and may play a significant role in community acquired CDIs in humans.

*SP-9*

## COMPARATIVE EXOPROTEOMIC OF BRAZILIAN *CLOSTRIDIOIDES DIFFICILE* RIBOTYPES TREATED WITH SUBINHIBITORY CONCENTRATIONS OF ANTIBIOTICS

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*Clostridioides difficile* is considered the major etiological agent of bacterial diarrhea associated with antibiotic use. Several studies have evaluated the impact of subinibitory concentrations of antibiotics on the *C. dificille* toxins expression. However, the interference of antibiotics on the expression of secreted proteins is poorly understood. Thus, the aim of this study was to identify and compare the exoproteome of exclusive brazilian *C. difficile* ribotypes, 133 and 135, with worldwide circulating ribotypes (RT) 014 and 027 (BI/NAP1), when grown under subinhibitory concentrations of clindamycin and levofloxacin. After the growth of ribotypes in the presence and absence of antibiotics, secreted proteins were obtained from all strains, followed by a digestion in solution, analysed and processed by the spectrometer Nano-LC ESI-MS/ MS coupled to LTQ Orbitrap. Approximately 290 proteins per condition and ribotype were obtained after analysis. All proteins were identified by using the Mascot software (data base Cdiff R20291) and thereafter validated by the Scaffold program. Analysis made by Blas2Go revealed that most of the proteins identified were related to cellular functions, such as transport, nutrient acquisition, adherence and some were associated with response to environmental stress. For the Brazilian ribotype 135, a protein that confers resistance to vancomycin and teicoplanin (gi|260686887 - vancomycin /teicoplanin A-type resistence protein), was identified only when the strain was subjected to subinibitory concentrations of antibiotics tested. This resistence phenotype (VanA) is described in Enterococci species for inducing high levels of resistance, which for *C. difficile* could be a problem for treating CDI. This study demonstrates the role of secreted proteins to adapt to environmental changes, and proteomics showed to be a useful approach in identification of new targets for functional analysis, and to better understand the biology of the main ribotypes associated with cases of infections caused by *C. difficile*.

## USING GENETICS TO UNCOVER THE ROLE OF TYPE 5 SECRETED AUTOTRANSPORTERS IN *FUSOBACTERIUM* NUCLEATUM VIRULENCE

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*F. nucleatum* is an anaerobic, Gram-negative bacterium that disseminates from its native oral cavity to contribute to multiple diseases, including a strong correlation with the development and progression of colorectal cancer (CRC). Interestingly, this bacterium lacks most major protein secretions systems aside from an overabundance of large Type 5 secreted proteins (autotransporters). To aid in virulence factor identification and characterization, an efficient and selectable genetic system has been developed in the Slade Lab that allows, for the first time, multiple markerless gene deletions and complementation of tagged proteins. Additionally, a novel suite of *in vitro* tools for studying autotransporters and bacterial cell surface proteins have been implemented to uncover the role of these virulence factors. Our recent completion of the *F. nucleatum* 23726 genome resulted in the correct annotation of the autotransporter protein family, including five genes from the Type Vc secreted trimeric autotransporters; herein renamed *Fusobacterium* Type Vc proteins (FvcA, FvcB, FvcC, FvcD, and FvcE). Using gene deletion strains, we reveal that monomeric (Type 5a) and trimeric autotransporters (Type 5c) are critical for host cellular invasion, and recombinant expression of these proteins in *E. coli* conferred an invasive phenotype from a previously non-invasive bacterium. In summary, we present an innovative set of genetic and molecular tools for *F. nucleatum* 23726 that has revealed a cooperative role for previously uncharacterized Type 5 secreted autotransporters in *F. nucleatum* virulence.


JULY 9-12, 2018
ANAEROBE 2018
Las Vegas, Nevada

***1300*** ***POSTER SESSION I:*** **ANIMAL STUDIES**



Posters will be presented in Poster Session I
Wednesday, July 11 1300-1400.

*PI-1*

## CULTIVATION OF FASTIDIOUS ANAEROBIC ORGANISMS FROM THE EQUINE GUT MICROBIOME USING THE ICHIP DEVICE FOR NON-TRADITIONAL CULTIVATION

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Cultivation is an invaluable tool in microbiology that allows for the characterization of an organism's morphological, physiological, biochemical, and chemotaxonomic traits. Currently, only a small fraction of microorganisms have been identified and described. The Ichip diffusion device is a non-traditional cultivation method developed to typically recover aerobic "uncultivable" organisms *in situ*. In this study, a culture-dependent approach was used to grow anaerobic fastidious microbes *in situ* using a modified Ichip device in order to identify novel anaerobic bacteria from the equine gastrointestinal tract. The principle behind this approach is that essential nutrients will migrate into the agar present in the Ichip device, further increasing the probability of continued growth when transferred to a range of substrates present in agar plate growth experiments. As a proof of concept, pre-constructed Ichip devices were inoculated with a diluted equine fecal sample and were placed in "environmental broth" in either an amended Brain-Heart Infusion (BHI) broth or a pure fecal slurry. Ichip devices were grown at 37˚C in anaerobic jars. After one week of incubation, the Ichip devices were deconstructed and the Ichip agar plugs were subcultured onto BHI + 5% Blood plates and incubated for one week at 37˚C. Colonies with diverse colony morphologies were subcultured to purity and identified via amplification of 16S rRNA gene. Preliminary results showed that the different "environmental broths" allowed for the growth of different taxa. In conclusion, the Ichip device, modified for anaerobic use, successfully allowed for the recovery of diverse taxa, demonstrating this cultivation method has the potential to encourage the growth of fastidious anaerobes. Ultimately, the Ichip device will be used to cultivate anaerobic bacteria from the fecal samples collected from a traditional Peruvian community. Subsequent testing will consist of modification of the Ichip for conversion into a microbial trap, with a larger pore bottom membrane, to allow for microbes to diffuse through and proliferate within the Ichip well.

# EVALUATION OF PREBIOTIC ADMINISTRATION IN LIPOPOLYSACCHARIDE-INDUCED SICKNESS BEHAVIOR IN MICE

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The brain-gut axis is a bi-directional pathway, where both organs influence each other's functions. Emerging evidence supports the two-way relationship between intestinal microbiota and psychiatric disorders such as autism, depression and anxiety. Some studies suggest that modulation of the microbiota by administration of prebiotics and probiotics might be an interesting approach to treat neuropsychiatric patients. The aim of the present study was to evaluate the anti-depressant effects of the prebiotic Inulin (0,001 mg/mL in drinking water) in behavioral models of depression. The three month-old male Swiss mice were treated with the prebiotic or vehicle for three weeks, before receiving a single i.p. injection of lipopolysaccharide (LPS; 0,83 mg/kg) and submitted to behavioral tests for evaluation of locomotion, memory, anxiety and depression. Animals given fluoxetine for three weeks prior to LPS administration were used as control. Stool collection was performed individually, at different periods throughout the experiment. Mice were sacrificed and the cecal content and intestines were collected for stool extraction. Pretreatment with the prebiotic decreased the depressive-behavior induced by LPS in mice. There was no influence of prebiotics on cognitive impairment and anxiety levels induced by LPS. Our current results show that the prebiotic presented anti-depressant activity. Subsequently, the PCR technique will be performed to quantify DNA of the main bacterial phyla present in the microbiota and the influence of the prebiotic on the microbiota alterations. Pro- and anti-inflammatory markers will be measured in brain and plasma of mice by ELISA, and brain levels of cathecolamines (noradrenaline, dopamine, serotonin) and their metabolites will be measured by HPLC.

Financial support: Capes, CNPq, FAPERJ

*PI-3*

## EFFECTS OF CHLORTETRACYCLINE AND DIETARY ZINC AND COPPER ON NURSERY SWINE *CLOSTRIDIUM DIFFICILE* CARRIAGE AND INTESTINAL MICROBIOTA

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*Clostridium difficile* is an anaerobic, spore forming bacteria that has the potential to be pathogenic, causing *C. difficile* infection in humans and animals. Antibiotics are used for the treatment, control, and prevention of infectious disease in agricultural animals. However, antibiotics disrupt the normal gut flora of the gastrointestinal tract, providing opportunity for *C. difficile* to proliferate in the intestines of swine. In farms, *C. difficile* is one of the major causes of diarrhea in piglets, which causes morbidity and ultimately leads to production losses. The objective of this study was to examine the effects of low and high levels of chlortetracycline, dietary zinc, and dietary copper on *Clostridium difficile* carriage and pathogenicity in nursery swine. Fecal samples were collected weekly from 126 pigs over the course of a 47-day study, and the experimental diets were arranged in a 2 x 2 + 2 factorial design. *C. difficile* was isolated from swine fecal samples by enrichment with cycloserine-cefoxitin-fructose broth, alcohol shock treatment, and cycloserine-cefoxitin-fructose agar restrictive media. Genomic DNA was extracted using the QIAamp DNA mini kit, and the *tcdA*, *tcdB*, and *cdtB* genes were amplified using previously published primers and protocols. Based on 192 samples evenly distributed between days 0, 14, 28, and 42, the prevalence of *C. difficile* carriage in this sample of nursery swine was 5-10%. The pathogenicity of isolated *C. difficile* based on PCR amplification revealed that all *C. difficile* isolates contained the *tcdA*, *tcdB*, and *cdtB* genes, and most were toxinotype V. Further metagenomic research will be conducted on fecal samples from swine fed dietary zinc and high chlortetracycline to observe the impact of treatment groups in relation to *C. difficile* carriage and swine microbial gut populations.

# EFFECT OF CEFTIOFUR AND CHLORTETRACYCLINE TREATMENT ON THE MICROBIAL GUT POPULATION OF SWINE EXPERIMENTALLY CHALLENGED WITH *SALMONELLA*

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Antibiotics are important in veterinary medicine to treat, control, and prevent infectious diseases; however, the contribution of antibiotic use in food animals to changes in the microbial gut population and potential food safety implications remain a topic of concern. The objective of this study was to investigate the effect of chlortetracycline and ceftiofur treatment on intestinal bacterial populations in swine challenged with *Salmonella*. A 2x2 factorial trial was conducted in two replicates with 32 pigs. In each replicate four pigs were assigned to each of the four treatment groups; chlortetracycline treatment, ceftiofur treatment, ceftiofur and chlortetracycline, and an untreated control group. Pigs were challenged on days 1 and 3 with a $10^4$ culture of *Salmonella enterica* transdermally and orally. A single dose of ceftiofur was given on day 5 and chlortetracycline was given in feed from day 5 to day 18. Intra-rectal fecal samples were taken daily throughout the study. Community DNA was extracted from fecal samples and used to conduct 16S rRNA metagenomics sequencing on the Illumina MiSeq platform following the recommend Illumina protocol. The most prevalent families found among the samples were Prevotellaceae, Ruminococcaceae, Veillonellaceae, Lachnospiraceae, and Clostridiaceae, respectively. The families identified between treatment groups or days did not differ significantly; however, the relative abundance of some families were significantly different based on two part statistical tests. The abundance of Clostridiaceae was significantly higher on days 11 and 18 compared to day 4. Interestingly, the abundance of Lactobacillaceae was significantly higher in the chlortetracycline/ceftiofur treatment group in comparison to the control group. Administration of antibiotics changes the relative abundance of bacterial families in the swine gut microbiota which may have a negative impact on swine health and food safety.


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## *1300* *POSTER SESSION I:* ANAEROBIC CLINICAL ISSUES IN HUMAN DISEASE



Posters will be presented in Poster Session I
Wednesday, July 11 1300-1400.

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Wednesday, July 11 1300-1400.

# THE *FIRMICUTES/BACTEROIDETES* RATIO IN THE GUT MICROBIOTA OF ADULT DIABETIC PATIENTS: A COMPARISON BETWEEN TYPE 1 AND TYPE 2 DIABETES

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**Purpose:** Our purpose was to investigate and compare the *Firmicutes / Bacteroidetes* ratio in the gut microbiota of Type 1 and Type 2 diabetes patients and healthy controls.

**Methods:** Between May 2016-April 2017, from fecal samples of 55 Type 2 diabetes patients and 55 healthy controls, the $\log_{10}$ *Bacteroidetes* and *Firmicutes* numbers were measured via qPCR and the *Firmicutes / Bacteroidetes* ratio was calculated and compared with that of Type 1 diabetes patient's (n:53) and healthy control's (n:53) measured from fecal samples collected between January 2014-October 2014, in our previous study. IBM SPSS statistics version 20 was used in statistical analyses.

**Results:** The *Firmicutes / Bacteroidetes* ratio was found as 1.236 ± 0.064 in Type 2 diabetes patients and 1.049 ± 0,074 in healthy controls. This ratio was 0,8029 ± 0,047 in Type 1 diabetes patients and 0,9134 ± 0,061 in healthy controls .

Comparing with those of Type 1 diabetes patients, the *Firmicutes / Bacteroidetes* ratio was found high in the gut microbiota of Type 2 diabetes patients ($p<0,0001$). Additionally, comparing with those of healthy controls, the *Firmicutes / Bacteroidetes* ratio was found high in the gut microbiota of Type 2 diabetes patients and low in Type 1 diabetes patients ($p<0,0001$).

**Conclusion**: Gut microbiota composition has been proposed to play an important role in the development of Type 1 and Type 2 diabetes. To explain this role we need to understand better a healthy normal gut microbiota and bacterial interaction. This requires large population studies with advanced techniques.

*PI-6*

## BRAIN ABSCESSES WITH ANAEROBIC BACTERIA: FIVE YEARS RETROSPECTIVE ANALYSES OF RESULTS

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**Purpose:** The etiology of brain abscess is usually polymicrobial involving aerobic and obligate anaerobic bacteria, therefore the investigation of anaerobes from brain abscess is of great importance. We reported the anaerobic bacteria isolated from brain abscesses and their frequency over a 5 year period.

**Methods:** Between January 2013 and January 2018, bacteriologic analyses were performed to 47 intra-operatively collected pus aspirate of brain abscesses. For the isolation of anaerobic bacteria, pus samples were inoculated onto anaerobic blood agar, phenyl ethyl alcohol blood agar, kanamycin vancomycin blood agar each prepared with Schaedler agar and 5% sheep blood and on fluid thioglycolate medium. Plates were incubated at $37^0$C for 5-7 days in anaerobic Gaspak jar (OXOID) Identification of anaerobic bacteria was done by Gram stain, aerotolerance test, by API 20A and VITEK 2 ANC cards (bioMérieux) from 2013to 2016 and by MALDI-TOF MS (Bruker) from 2016 to 2018.

**Results:** Using appropriate microbiological techniques, 36 anaerobic bacteria strains were isolated in 24 of 47 patients (51%). Anaerobic bacteria were associated with aerobic strains in 8 patients (17%), whereas in 16 patients (34%) only anaerobic strains were isolated. The most frequently isolated species were *Peptospstreptococcus anaerobius* (n:14), *Bacteroides fragilis* (n:8), *Prevotella* spp. (*n*=5), *Fusobacterium nucleatum* (n:3), *Propionibacterium acnes* (n:3), *Bacteroides thetaiotaomicron* (n:1), *Parvimonas micra* (n:1), *Veillonella parvula (n:1)*

**Conclusions:** Anaerobic bacteria are common in brain abscess and should always be considered while treating a brain abscess. A high degree of suspicion, proper microbiological techniques for isolation of organisms, and the underlying risk factor would help in the management of these patients.

# ANTIBIOTIC SUSCEPTIBILITY PROFILES OF RECENT ANAEROBIC BACTERIAL ISOLATES FROM EUROPE

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**Objective:** Anaerobic infections tend to be polymicrobial, and are often treated empirically with broad-spectrum therapies. Susceptibility to the most commonly used antimicrobials among anaerobic pathogens varies among genera and even the species within a genus. Difficulty in isolating primary anaerobic pathogens and limited susceptibility testing by microbiology laboratories often results in the use of empirical treatment. For this reason, it is important to provide surveillance data to guide providers in the most effective choices for anti-anaerobic therapy. In this study, we report the susceptibility data for recent anaerobic clinical isolates from European hospitals from 2015 through 2017 collected through a surveillance study.

**Methods:** 2,739 anaerobic pathogens (1,872 Gram negative isolates, 867 Gram positive isolates) were collected from 2015-2017 from 19 sites in 7 countries in Europe (Belgium, Czech Republic, France, Germany, Hungary, Spain, and Sweden). Gram negative organisms included *Bacteroides* spp., *Parabacteroides* spp. and *Prevotella* spp. Gram positive organisms included anaerobic cocci and *Clostridium* spp. excluding *C. difficile*. Organism identification was confirmed at a central laboratory (IHMA, Inc., Schaumburg, IL, US) by MALDI-TOF mass spectrometry and MIC values were determined using agar dilution following CLSI guidelines. Percent susceptible (%S) was calculated using CLSI breakpoints for cefoxitin (FOX), clindamycin (CLI), meropenem (MEM), metronidazole (MTZ), penicillin (PEN) and piperacillin-tazobactam (TZP), and FDA breakpoints for tigecycline (TGC).

**Results:** %S for *B. fragilis* group was: MTZ, 99.9; TZP, 97.4; MEM, 97.1; TGC, 88.5; FOX, 75.9; CLI, 57.1. %S for *Prevotella* spp. was: MTZ, 100; TZP, 100; MEM, 99.9; FOX, 98.8; TGC, 98.8; CLI, 67.7. %S for anaerobic cocci was: MTZ, 100, TGC, 100; MEM, 99.8; TZP, 99.8; PEN, 97.7; CLI, 77.3. %S for *Clostridium* spp. was: MTZ, 100; MEM, 100; TGC, 99.8; TZP, 99.8; PEN, 80.1; CLI, 72.8.

**Conclusions:** Metronidazole, meropenem, and piperacillin-tazobactam, and tigecycline, showed excellent *in vitro* activity against all anaerobic organisms isolated from European hospitals, with >97% susceptible, with the exception of tigecycline against *Bacteroides* spp. (88.5% susceptible). Clindamycin, penicillin, and cefoxitin exhibited lower activity overall, with less than 68% of gram negative isolates susceptible to clindamycin. Cefoxitin was active against *Prevotella* spp. but only inhibited 76% of *Bacteroides* spp. Differences in susceptibilities between genera highlight the need for continued evaluation of antimicrobial susceptibilities of anaerobic organisms to aid in the selection of empiric therapy and monitor resistance trends.

*PI-8*

## SEX-SPECIFIC DIFFERENCES IN THE INCIDENCE OF *FUSOBACTERIUM NUCLEATUM* SUBSPECIES IN THE ORAL CAVITY

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The periodontal pathogen *Fusobacterium nucleatum* (FN) has been implicated in several extra-oral diseases, including preterm birth and colorectal cancer. Due to its genetic and phenotypic heterogeneity, FN is classified in four subspecies which may differ in their disease potential. Here we compared the prevalence of FN subspecies and the close relative *F. periodonticum* (FP) via 16S rRNA gene analysis in saliva from 100 healthy individuals (60 females, and 40 males) from eleven countries spanning five continents. By focusing on the most abundant sequence types (i.e. analysis of approximately ten clone sequences each) the average number of FN/FP subspecies per individual differed significantly between females and males, i.e. 2.93 versus 2.5, respectively (p=0.043). FN subsp. *fusiforme/vincentii* was significantly more prevalent in females vs males, with 2.85 vs. 1.68 sequence reads per individual, respectively (p=0.012). A significant age-related difference was observed in females but not in males, i.e. 2.6 subspecies on average in young females ≤30 years vs. 3.2 in older females >30 (p=0.0076). Given the link between FN and systemic disorders our findings highlight the need for microbial studies at the subspecies level to further characterize the role of periodontal pathogens in diseases that affect females and males differently, e.g. colorectal cancer.

## SPECTRUM OF ANAEROBIC INFECTIONS IN A TERTIARY CARE HOSPITAL

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Anaerobic bacteria are known to cause predominantly subcutaneous and deep seated infections, gas gangrene being the major one. Most of these infections are polymicrobial in association with aerobic flora. Anaerobes being difficult to grow often neglected during bacteriological diagnosis. The fastidious nature of anaerobes compel for appropriate sample collection and transportation of the specimen. Requirement of anaerobiosis and slow growing nature of anaerobes make their isolation more tedious. The necessity of isolation and identification of anaerobic organism increases with the reports of acquiring resistance to the commonly used antibiotics. Usually a combination of antibiotics covering aerobic and anaerobic flora is given in such infections. Considering the dominance of anaerobes in certain infections for example deep seated abscesses and gas gangrene, isolation of anaerobes along with sensitivity pattern can lead to better management of patient. So, the present study was planned to isolate anaerobic organisms from various deep seated and subcutaneous infections to know the prevalence and antibiotic sensitivity pattern.

This was a prospective laboratory based study which included 118 clinical samples suspected of anaerobic infections over the period of 2 years. Samples were processed by standard microbiological methods for identification of anaerobic bacteria and were further confirmed by PCR and MALDI-TOF. Antibiotic susceptibility testing was done by CLSI guidelines.

Out of total 118 clinical samples 89 (75.42%) samples were from suspected gas gangrene cases and remaining 29 (24.57%) samples were from deep seated infections. In 15 (12.71%) samples anaerobic bacteria were isolated of which 6 (40%) were *Clostridium perfringens and Clostridium spp.* each and 3 (20%) were *Peptostreptococcus spp* and all these isolates were confirmed by PCR and MALDI-TOF.

Deep seated and subcutaneous infections should also be screened for anaerobic infections along with aerobic flora which will decrease morbidity and mortality.

*PI-10*

## BLOODSTREAM INFECTIONS AND ANTIBIOTIC SUSCEPTIBILITY OF ANAEROBES ISOLATED FROM ORTHOPAEDIC PATIENTS

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Blood culture for anaerobes is not carried out routinely in many hospitals in Nigeria. The implication is that anaerobes are not usually considered in treatment of infection that may be polymicrobial as may be seen in bloodstream infections. Twelve patients that developed pyrexia following sepsis of orthopedic wounds (hip joints, prosthetic joints and fractured jaw) were investigated in a University Teaching hospital in Lagos, Nigeria. Blood cultures revealed mixed infections with aerobes and anaerobes. Coagulase negative staphylococci, methicillin sensitive *Staphylococcus aureus*. *Pseudomonas aeruginosa* and *Klebsiella* species were the aerobes isolated. *Finegoldia magna* was isolated from two patients with PJI and a patient with hip joint infection. *Bacteroides fragilis* was isolated from two patients, one with PJI and the other hip joint infection. *Prevotella intermedia* and *P. melaninogenica* were isolated from two and one patient with fractured jaw sepsis respectively. Blood cultures were positive only for *B. fragilis* and *P. intermedia*. The anaerobes were tested against penicillin, amoxicillin, amoxicillin-clavulanic acid, cefoxitin, moxifloxacin, metronidazole and tigecycline. *Finegoldia magna* wound and blood isolates were sensitive to all the antibiotics. *Prevotella* species both wound and blood isolates were sensitive to the antibiotics except pencillin, and amoxicillin. One of the two *B. fragilis* from blood was resistant to the β-lactam antibiotics, and tigecycline. The other *B. fragilis* isolate from blood was sensitive to tigecycline in addition to metronidazole and moxifloxacin. All *B. fragilis* wound isolates were resistant to the β-lactam antibiotics except cefoxitin, but sensitive to metronidazole, moxifloxacin and tigecycline. The difference in antibiotic susceptibility of blood and wound isolates highlights the need for full anaerobic investigation of septicemia originating from a local sepsis for which anaerobes are isolated.

# THE MOLECULAR MECHANISM UNDERLYING CARBAPENEM RESISTANCE IN *BACTEROIDES FRAGILIS*

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Antimicrobial resistance is growing seriously, which makes it critically important for laboratories to closely monitor the trend and mechanisms of emerging antibiotic resistance in clinical pathogens. However, the surveillance of antibiotic resistance in anaerobes and the relevant mechanistic study are lacking in China. This study investigated the molecular mechanism underlying carbapenem resistance in 44 strains of *Bacteroides fragilis* isolated from Huashan Hospital, Fudan University during the period from January 2009 to December 2015. Antimicrobial susceptibility testing showed that among the 44 *Bacteroides fragilis* isolates, 18.2%, 29.6%, 22.7%, 100%, 100%, 29.6%, 15.9%, 81.8%, 88.6%, and 47.7% were resistant to imipenem, meropenem, ertapenem, penicillin, ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, clindamycin, tetracycline, and moxifloxacin, respectively. None of the *Bacteroides fragilis* strains was resistant to metronidazole, cefoxitin or chloramphenicol. The *cfiA* gene encoding metallo-beta-lactamase was identified in 16 of the 44 *Bacteroides fragilis* isolates, including 8 strains with insertion sequence in the upstream and high level resistance to carbapenems. Southern hybridization experiment demonstrated that *cfiA* gene was all located in chromosome. Bioinformatic analysis revealed that the upstream insertion sequence was primarily IS1187 (6 strains) and IS613 (2 strains). Relative quantification of qRT-PCR indicated that IS may mediate the resistance to carbapenems in *Bacteroides fragilis* via upregulating *cfiA* expression level. This is the first study in Mainland China to explore the molecular mechanism of carbapenem resistance in *Bacteroides fragilis*. The prevalence of carbapenem resistance is much higher in our study than the reports from other countries or regions. Chromosome-derived *cfiA* is the main molecular mechanism mediating carbapenem resistance in *Bacteroides fragilis*.

*PI-12*

## A CASE OF MULTI-DRUG RESISTANT *BACTEROIDES FRAGILIS* IN A POLYMICROBIAL LIVER ABSCESS

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We describe a challenging case of multi-drug resistant (MDR) *Bacteroides fragilis* in a patient with significant comorbidities. A 64-year-old Kuwaiti national presented to a London hospital in May 2017. She had a background of metastatic pancreatic cancer, type 2 diabetes, sarcoidosis and asthma. She was known to be colonized with methicillin-resistant *Staphylococcus aureus*. Five days earlier she had undergone chemotherapy via her implanted central venous catheter (PortaCath). She presented with constipation, pyrexia and drowsiness on admission. A right sided pleural effusion was noted and a pleural drain was inserted. Admission blood cultures grew *Klebsiella oxytoca* and *Serratia marcescens* (sensitive to ciprofloxacin, gentamicin and trimethoprim on disc diffusion) and she was switched from co-amoxiclav to ciprofloxacin and gentamicin. She continued to be febrile with increasing inflammatory markers. On day 4 of admission pleural fluid grew *Bacteroides fragilis*. Whilst awaiting reference laboratory sensitivity testing, meropenem and linezolid was started. Further blood cultures grew *Bacteroides fragilis*, with the same sensitivity pattern (resistant to metronidazole (MIC >256) clindamycin, co-amoxiclav, meropenem, piperacillin-tazobactam, ceftriaxone, linezolid, moxifloxacin and chloramphenicol, sensitive to tigecycline). A further blood culture grew *Serratia marcescens*. Tigecycline was added on day 4 of meropenem and linezolid. Unfortunately, she deteriorated further and became neutropenic. On day 7 of meropenem, tigecycline and linezolid, a CT abdomen revealed new lesions compatible with liver abscesses. Her family declined PortaCath removal and repatriated her to Kuwait for palliation. The delay to appropriate antibiotics due to ineffective empirical therapy pending reference laboratory testing likely led to her poor response. A significant disease burden and polymicrobial infection were additional factors. Case reports of MDR *Bacteroides fragilis* remain rare, particularly with this profile. Although metronidazole is the mainstay of empirical treatment, an increase in cases like this highlights the need for vigilance and lower threshold for extended sensitivity testing.

# CLINICAL INVESTIGATION OF *BACTEROIDES* BACTEREMIA IN A TERTIARY HOSPITAL

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**Objective** *Bacteroides* spp. are obligate anaerobic Gram-negative rods which are important in intraabdominal infections, post-surgical wound infections, and bacteremia; however, *Bacteroides* infections have not been adequately studied in Japan. The objective of this study was to assess the clinical characteristics of *Bacteroides* bacteremia.

**Methods** A retrospective study was performed, and data regarding the clinical background, microorganisms detected, antimicrobial susceptibility, treatment and prognosis were analyzed in 43 cases of bacteremia caused by *Bacteroides* spp. in Osaka City University hospital from January 2007 to October 2015.

**Results** There were 25 men and 18 women; average age was 61.1 years (20-80 years). The most common underlying disease was malignant tumor (26 cases, 60.5%); of these eight were gynecological tumors (18.6%), and 7 were colorectal tumors (16.3%). The most commonly detected microorganism was *B. fragilis*, followed by *B. thetaiotaomicron*. The portal of entry was via intraabdominal infections in 16 cases (37.2%), but could not be confirmed in about half of the cases. Antimicrobial susceptibility was SBT/ABPC: 94.4%, TAZ/PIPC: 94.4%, CMZ: 80%, CLDM: 70%, IPM: 97.5% and MEPM: 94.4%, which was comparatively good, except in the case of CLDM; however, one case was of a multiple drug resistant strain, including carbapenem resistance. The most common empiric therapy was with carbapenem (24 cases, 55.8%), followed by CMZ (6 cases, 14.0%). TAZ/PIPC and MNZ were each selected as initial treatment in only one case (2.3%). Four patients (9.3%) died within 30 days of developing bacteremia; 10 patients (23.3%) died in hospital.

**Conclusions** The results for antimicrobial susceptibility and the prognosis of bacteremia due to *Bacteroides* spp. in our hospital were similar to the results in previous reports; however, carbapenem was the most frequently chosen initial treatment, and resistant organisms were detected. From the viewpoint of antimicrobial stewardship, we should reconsider the selection of appropriate antimicrobial agents for initial therapy.

*PI-14*

## ASSOCIATION BETWEEN ONCOGENIC BACTERIA AND COLORECTAL CANCER

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Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world. Several risk factors that may increase CRC include old age, polyps, high consumption of red meat, obesity, smoking, alcohol intake, and dysbiosis of gut microbiota.The association between oncogenic bacteria, including *Escherichia coli* possessing colibactin, *Enterococcus faecalis,* enterotoxigenic *Bacteroides fragilis* (ETBF), *Fusobacterium nucleatum* and *Streptococcus gallolyticus*, and CRC has been established over the past few decades. However, the mechanisms underlying the bacterial contribution to CRC development are not yet clear. We determined the presence of five oncogenic bacteria (*clbB*-positive *E. coli, E. faecalis*, ETBF, *F. nucleatum*, and *S. gallolyticus*) in paired carcinoma tissue (CT) and adjacent normal tissue (AT), as well as in age-matched healthy control in South Korea. Colon tissues of 35 CRC cases were collected from paired CT and AT during colonoscopy. Normal colon tissues (NT) of 24 controls were obtained from paired left-colon and right-colon. The presence and abundance of oncogenic bacteria was examined using real-time PCR and culture. We found that the presence of *F. nucleatum* was strongly associated with CT of cases compared to NT of controls (82.9% vs. 12.5%, $p < 0.0001$). The adhesin gene of *F. nucleatum*, fadA, was significantly higher in CT than that detected in NT by PCR (71.4% vs. 8.3%; $p < 0.0001$). However, no association was seen with the presence of ETBF, *clbB*-positive *E. coli, E. faecalis* and *S. gallolyticus*. In conclusion, *F. nucleatum* is more prevalent in CT and AT of CRC cases than in NT of controls and fadA is also prevalent in CT. Our results support the hypothesis that *F. nucleatum* is a causal factor in CRC development.

# *CUTIBACTERIUM ACNES* IN PROSTHETIC JOINT INFECTION

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Joint replacement is an important strategy for treating various musculo-skeletal conditions, but a group of patients will suffer failure within 10 to 15 years following installation of those devices. Among the anaerobic microorganisms isolated in those infections, *C. acnes* is the most prevalent. Hence, this study aimed to evaluate *C. acnes* strains isolated from clinical specimens of patients with orthopedic implants. A total of 66 inpatients from the National Institute for Trauma and Orthopedic (INTO) located in Rio de Janeiro, Brazil, were included in this study. During surgery, tissue fragments and fluids were removed and placed into tubes containing thioglycollate broth and glass beads, shaken for 30 sec and seeded onto solid blood agar plates. Implants were placed in sterile rigid vials with 500 mL of lactated ringer's solution and mechanically shaken for 1 min. Four aliquots of 50 mL were centrifuged for 15 min and pellets seeded onto blood agar plates and into thioglycollate. All plates and broths were incubated for 14 days at 37°C under anaerobic conditions and the broths were subcultivated within this period. Of 66 patients, 73% were recovered from hip arthroplasty, 18% from knee arthroplasty, 8% from shoulder implants and 2% from spine implants. *C. acnes* accounted for 11 patients (16.66%) and most of the strains were recovered in the 7$^{th}$ and 14$^{th}$ days of incubation. In only 3 patients, infection was not confirmed. All strains were strongly biofilm producers, 4 were beta-hemolytic and any of them produced CAMP factor. All of them were sensitive to all evaluated antimicrobials. In conclusion, in our study *C. acnes* was the most recovered anaerobic microorganism from orthopedic implants. Our results reinforce the importance of implementing a routine for researching anaerobic microorganisms in infections associated with orthopedic implants.

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*PI-16*

## A 4-YEAR SURVEY OF ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF ANAEROBIC BACTERIA ISOLATED FROM PATIENTS WITH BLOODSTREAM & WOUND INFECTIONS IN A TERTIARY CARE HOSPITAL OF SOUTH ITALY

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**Objective:** In the present study, we retrospectively reviewed the microbiological records of blood samples received for anaerobic culture, January 2014 to December 2017, at our tertiary care referral hospital in Southern Italy. We also analyzed the distribution of anaerobic bacteria isolates obtained from different types of purulent wound infections.

**Methods & Results:** 18,.893 blood samples were received in our laboratory. Anaerobes were identified by standard biochemical tests (blood samples cultured using BACTEC Plus Anaerobic/F Culture Vials [Becton Dickinson Italia s.p.a.]; wound samples using BacT/ALERT SN Anaerobic [BioMerieux Italia s.p.a.]. The isolated colonies are subjected to identification with VITEK2 or VitekMS (BioMerieux Italia S.p.A.) and antimicrobial susceptibility in Microdilution (API-BioMerieux Italia S.p.A.) or by Kirby-Bauer on Agar Mueller Hinton Blood (BioMerieux Italia S.p.A.). A total of 3,896 bacterial isolates were obtained from blood cultures; of these, 25 (0.66% of all positive blood cultures) were positive for anaerobes. The mean percentage of positive cultures for anaerobic bacteria/total cultures required was 0.16; the mean percentage of positive cultures for anaerobic bacteria/total positive cultures was 0.64.

Comparison of cumulative susceptibility of anaerobic bacteria isolates from blood and wound infections cultures to antibiotics: Imipenem 100%, Metronidazole 95.7%, Ticarcillin/clavunate 95.7%, Piperacillin/tazobactam 91%, Amoxicillin/clavulanate 91.7%, Vancomicin 83.3%, Clindamycin 70.8%, Piperacillin 66.7%, Ticarcillin 43.5%, Amoxicillin 37.5%, Benzylpenicillin 25%.

**Discussion:** In the present study over four years, the overall isolation rate of anaerobes in blood was found to be 0.66%. Anaerobic bacteria isolated from blood and wound infections showed increased resistance to penicillins. It is of interest the reduced sensitivity to vancomycin and especially to clindamycin. The results indicate an excellent sensitivity to imipenem and metronidazole and a good sensitivity to penicillins with beta-lactamase inhibitor. Significant incidence of anaerobic bacteraemia and wound infections, emerging antimicrobial resistance in anaerobes to commonly used anti microbials suggest a routinely complete investigation of all blood and wound infections cultures for anaerobes, as part of an antibiotic stewardship program.

# DETECTION OF THE MAIN ANTIBIOTIC RESISTANCE GENES OF *BACTEROIDES* AND *PARABACTEROIDES* STRAINS ISOLATED FROM THE INTESTINAL MICROBIOTA OF HUNGARIAN PATIENTS

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We aimed to record the antibiotic susceptibilites for intestinal isolates to see the situation, to compare with clinical strains and get information for the source of clinical strains.

Intestinal *B. fragilis* group strains (n=92, 14 *B. fragilis* and 78 non-fragilis *Bacteroides*) were isolated from stool samples of healthy and carbapenem-treated patients in Hungary using the novel Chromogenic *Bacteroides* Agar (CBA). Antibiotic susceptibilities were determined by agar dilution and the presence of eight antibiotic resistance genes (*cepA*, *cfxA*, *cfiA*, *erm*(*F*), *nim*, *bex*A, *tetQ* and *tetX*) were detected by previously established RT-PCR methods.

For the eight genes examined we got the following data: *cepA* was *B. fragilis*-specific and 11 *B. fragilis* strains (78.6%) were positive for it while 3 *B. fragilis* strains were positive for *cfiA* (21.4%, all from healthy patients), the prevalence for *cfxA*, *erm*(*F*), *nim*, *bexA*, *tetQ* and *tetX* were as follows: 60.9, 54.4, 0, 16.3, 73.9 and 45.7%. The frequencies of *cfxA*, *erm*(*F*), *bexA* and *tetX* were statistically higher in the normal flora group with the following p values $<0.001$ (*cfxA*, *erm*(*F*) and *tetX*) and 0.032 (*bexA*) while the frequencies for *cfiA*, *nim* and *tetQ* were the same in the two groups. The occurrence of *erm*(*F*) and *tetX* higly correlated ($r=0.842$, $p<0.001$) indicating common genetic carrier(s).

Because of the higher prevalence of some genes in the normal flora group we hypothetize that there are two different *Bacteroides* populations in the gut, e.g. one the luminal from which the stool samples may originate and second the mucosa associated population from which the infections may originate. Additionally, the intestinal *Bacteroides* population may be a reservoir for shedding antibiotic resistance genes.

This study was supported by the ESCMID Study Group on Anaerobic Infections.

*PI-18*

## EPIDEMIOLOGY OF MOXIFLOXACIN SUSCEPTIBILITIES AND THE CARRIAGE OF MULTIDRUG EFFLUX PUMP GENE OF HUNGARIAN *BACTEROIDES* ISOLATES

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**Objective:** The different multidrug efflux pumps are important resistance mechanisms to fluoroquinolones. One of these mechanisms is the *bexA* gene, which is a Multidrug And Toxic compound Extrusion (MATE) pump family member. We aimed to examine the moxifloxacin susceptibilities and the prevalence of the *bexA* gene in *B. fragilis* group isolates from Hungary.

**Methods:** Antibiotic susceptibility test was performed for moxifloxacin for 400 different Hungarian *B. fragilis* group isolates (233 *B. fragilis*, 167 non-fragilis *Bacteroides* (NFB)) by the agar dilution method. The presence of the *bexA* gene was investigated in 92 *B. fragilis* group isolates (46 *B. fragilis* group strains with moxifloxacin MIC value of 8 - ≥32 µg/ml and 46 isolates with MIC of ≤2 µg/ml) by RT-PCR method. The data were analyzed by chi-squared tests.

**Results:** We found that 18.5% of all strains was resistant to moxifloxacin (15.45% of the *B. fragilis*; 22.75% of the NFB strains). The results indicate statistically significant (p=0.004) increasing moxifloxacin resistance rate in comparison with European resistance data published earlier. We detected the presence of the *bexA* gene in 10 strains from the group of elevated moxifloxacin MICs (8 - >32 µg/ml) and 9 isolates form the susceptible group (MIC value of ≤2 µg/ml). However, comparison with earlier data, these prevalences are higher. The *bexA* gene prevalence was significantly higher (p=0.002) in NFB strains than in *B. fragilis* isolates.

**Conclusion:** We found that the rate of moxifloxacin resistance strains, the prevalence of *bexA* gene are increasing and more NFB isolates harboured this gene. The presence of activating IS-element should also be investigated to reveal their role in strains with elevated moxifloxacin MIC-values.

# ANTIMICROBIAL SUSCEPTIBILITY OF *PREVOTELLA* SPECIES FROM TWO CENTERS IN TURKEY AS DETERMINED BY E-TEST METHODOLOGY

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Knowledge about the antimicrobial susceptibility patterns of different *Prevotella* species is limited in Turkey. Providing antimicrobial susceptibility data of these bacteria is very important for effective empirical treatment.

**Objective:** We aimed to determine susceptibility data for 12 antimicrobial agents against *Prevotella* strains originating from human infections, collected in two centers in Turkey.

**Methods:** In total 118 *Prevotella* species isolated from different clinical samples, collected between January 2015 and December 2017, were tested. Organisms were identified by using MALDI-TOF MS and by 16S rRNA gene sequencing. MICs of 12 antibiotics were determined using E-test methodology (bioMerieux, France) and the EUCAST, CLSI and FDA guidelines were used for interpretation.

**Results:** *Prevotella nigrescens* was the most prevalent species (n=20) followed by *P. buccae* and *P. bivia*. All *Prevotella* strains, representing 12 different species, were susceptible to piperacillin/tazobactam, imipenem, meropenem, tigecycline and cefoxitin. A total of three (2.5%) isolates were non-susceptible to metronidazole and two (1.7%) isolates to ampicillin/sulbactam. The proportion of non-susceptible isolates to ampicillin, clindamycin, tetracycline and moxifloxacin were 50.8%, 32.2%, 23.7% and 16.9%, respectively.

**Conclusion:** Piperacillin/tazobactam, cefoxitin, and tigecycline display high in vitro activity against *Prevotella* spp. and they all remain good candidates for empiric therapy. Imipenem and meropenem were also found to be very active, but the usage of carbapenems should be reserved for serious mixed infections, potentially accompanied by other resistant organisms. Non-susceptibility to ampicillin-sulbactam and metronidazole emphasize the need of periodic monitoring of their susceptibility patterns. The high rates of non-susceptibility to ampicillin, clindamycin, tetracycline and moxifloxacin indicate that these antimicrobials should not be used for empirical treatment of infections without prior antimicrobial susceptibility testing.

*PI-20*

## CASE REPORT 1: BACTEREMIA DUE TO BUTYRICIMONAS SPECIES IN AN IMMUNOCOMPROMISED PATIENT

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Anaerobic bacteraemia mostly originates from endogenous microbiota . Mortality rate of blood circulation infections is high and early diagnosis and appropriate treatment are critical.

**Case Presentation:** A 48-year-old woman admitted to the internal medicine department with a history of pancytopenia and chronic hepatitis C virus and cytomegalovirus infections. The patient, underwent kidney transplantation 12 years ago, received immunosuppressive medication, prednisone, and antiviral therapy. On the day 32 of therapy, she developed fever, abdominal pain, nausea, and vomiting with abdominal rigidity and tenderness. Abdominal computed tomography scan revealed diffuse fluid level. Considering these findings, the patient was diagnosed with intestinal perforation. Two set of blood samples were collected for culture. Laparotomy was performed immediately. The sigmoid colon was resected with closing of the rectal stump and colostomy. Vancomycin, meropenem were administered empirically. The patient was followed up in intensive care unit, however she died 17 days later of this operation.

Gram-negative rods, isolated from anaerobic blood culture bottles after a 72h incubation period, were obligate anaerobic, catalase producing, inhibited on Bacteroides bile aesculin agar, resistant to vancomycin, kanamycin and colistin sulfate suggesting that the isolate was a *Prevotella* species. However; both Rapid ID 32A and VITEK MS (bio Mérieux- France) were insufficient for identification. The 16S rRNA gene sequencing showed 98% nucleotide identity to those of strains *Butyricimonas faecihominis* and *B. paravirosa* isolated from human faeces (GenBank, NR_126194 and NR_126195, respectively). Our strain was mannose and rafinose fermentation reactions positive, however both reactions were negative for *B. paravirosa*, mannose negative for *B. faecihominis*. Antibiotic susceptibility results, by E-test methodology, showed sensitivity to beta-lactam/beta-lactamase inhibitor combinations, carbapenems, metronidazole and tigecycline, however resistance to amoxicillin, cephalosporins, clindamycin, tetracycline and moxifloxacin.

We suggested that the isolate was part of the patient's intestinal microbiota and her underlying diseases had created predisposition for bacteremia. Bacteremia due to *Butyricimonas* species is extremely rare and few are known about pathogenesis. Our strain might be a new *Butyricimonas* species and Identification of all the features are needed.

# CASE REPORT 2: POLYMICROBIAL ANAEROBIC MENINGITIS CAUSED BY *BACTEROIDES FRAGILIS*, *BACTEROIDES THETAIOTAOMICRON*, *FUSOBACTERIUM NECROPHORUM* AND *SLACKIA EXIGUA* IN A PATIENT WITH MASTOIDITIS FOLLOWING OTITIS MEDIA INFECTION

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Anaerobic meningitis may occur secondary to spread of infection from mastoiditis, an intratemporal complication of otitis media. The prognosis of anaerobic meningitis is usually grave, and the mortality rate is high. Early recognition and proper therapy may allow survival.

**Case presentation:** A 16-year-old male, who presented with fever and headache admitted to emergency department of Marmara University hospital. The patient diagnosed meningitis according to abnormal CSF profile; white cell count 9000/mL and the presence of leukocyte and gram negative bacteria in the stained preparation. Vancomycin, meropenem and fluconazole were administered empirically. The patient was transferred to another hospital due to lack of room in our hospital. CSF specimen was inoculated onto blood and chocolate agar plates and incubated at 35°C in an environment with 5% CO2. In addtion, thioglycolate broth was used to allow for recovery of anaerobes and organisms that may be present in low numbers in a specimen. Although there was no growth on the solid media, turbidity was observed in the lower part of the thioglycolate broth. Subculture was performed from liquid medium for aerobic and anaerobic organisms and incubated at 35°C in an environment with 5% CO2 and in anerobic chamber, respectively. The organisms were only growth on anaerobic blood agar. Four different type colonies were observed and three types of them were identified by MALDI TOF MS as *Bacteroides fragilis, B. thetaiotaomicron* and *Fusobacterium necrophorum*. The fourth organism showed 99% nucleotid identity to *Slackia exigua* (NR_024952.1) by using 16S rRNA gene sequencing. Antimicrobial susceptibility tests of organisms were performed by using E-test methodology. Since the meropenem MIC value of *B. fragilis* was 3 mg/L (inremediate; accordig to EUCAST breakpoint value), metronidazole was added to antimicrobial therapy. The patient applied to our hospital for control examination after two months, and left-sided peripheral facial paralysis, due to complication of left mastoidectomy operation, was detected.

**Conclusion:** Anaerobic culture of cerebrospinal fluid (CSF) specimens is essential to recover the anaerobic pathogens. Anaerobes can be identified rapidly and reliably by using MALDI TOF MS technology. Identification of organisms at species level and testing for antibiotic susceptibility in rouitine microbiology laboratory will be life-saving.

*PI-22*

# INVESTIGATION OF β-LACTAMASE ACTIVITY OF CLINICAL *PREVOTELLA* ISOLATES BY USING COMBINATION DISK METHOD

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Penicillins are most frequently used in the empirical treatment of infections caused by *Prevotella* species. Resistance to penicillins occurs mainly due to β-lactamase production. In routine laboratories, β-lactamase production is mostly determined by using a nitrocefin test, however, since pigmented *Prevotella* species give indistinguishable results, evaluation of β-lactamase activity is difficult.

**Objective:** We aimed to determine β-lactamase activity of *Prevotella* species by antibiotic combination discs, which used for detection of ESBL positive aerobic bacteria.

**Methods:** In total 70 *Prevotella* species isolated from different clinical samples, collected in two Turkish university hospitals, were tested. MICs of ampicillin and ampicillin/sulbactam were determined using E-test methodology (bioMerieux, France) and the EUCAST guideline was used for interpretation. Extended β-lactamase activity was investigated by using discs containing cephalosporins alone (cefotaxime and ceftazidime) and in combination with clavulanic acid. The test was positive if the inhibition zone diameter around the cephalosporin disk with clavulanic acid was ≥5mm larger than without. Brucella medium (BD Difco, USA) was used and bacterial suspension was match a density of 1 McFarland in both test procedures. β-lactamase activity coding gene (*cfxA*) detected by PCR method.

**Results:** All isolates were susceptible to ampicillin/sulbactam. The $MIC_{50}$ and $MIC_{90}$ values and resistance rate were 1, >256 mg/L and 57.1%, respectively, for ampicillin. Of all isolates, 77.1% were cfxA gene and 54.2% were ESBL positive. All ESBL positive isolates were *cfxA* gene positive.

**Conclusion:** Our data indicate that, the high level of resistance to penicillin, which may be used for treatment of infections involving *Prevotella* species, is due to β-lactamase activity. For detection of β-lactamase activity, the combination disk test, a simple and reliable method, can be used in routine microbiological laboratories.

# ANTIMICROBIAL SUSCEPTIBILITIES FOR ANAEROBIC GRAM-POSITIVE COCCI ISOLATED IN JAPAN

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**Objectives:** To investigate current status of antimicrobial resistance for anaerobic gram-positive cocci in Japan.

**Methods:** We investigated antimicrobial susceptibility of 16 antibacterial agents (ampicillin (ABPC), amoxicillin/culavulanate acid (AMPC/CVA), piperacillin/tazobactam (PIPC/TAZ), cefmetazole (CMZ), flomoxef (FMOX), ceftazidime (CAZ), cefoperazone/sulbactam (CPZ/SBT), cefozopran (CZOP), imipenem (IPM), meropenem (MEPM), clindamycin(CLDM), minocycline (MINO), levofloxacin (LVFX), moxifloxacin (MFLX), garenoxacin (GRNX), sitafloxacin (STFX) ) for 102 strains of *Parvimonas micra,* 93 strains of *Peptostreptococcus anaerobius*, 83 of *Finegoldia magna,* 76 of *Peptoniphilus asaccharolyticus* isolated in Aichi Medical University Hospital, Japan, from 2011 to 2017. Antimicrobial susceptibility was measured using dry plate methods (Dryplate-eiken, Eiken Biological Technology Co. Ltd., Tokyo, Japan) based on microdilution method. Antimicrobial resistance was fundamentally judged by Clinical and Laboratory Standard Institute (CLSI) criteria.

**Results:** CMZ, PIPC/TAZ, IPM and MEPM showed relatively good antimicrobial activity for anaerobic gram-positive cocci investigated. Susceptible rates of NIMO against *P. micra*, *P. anaerobius, F. magna* and *P. asaccharolyticus* were 91.7%, 87.8%, 91.5% and 82.7%, respectively. Susceptible rates of CLDM remained 91.7%, 52.5%, 67.1% and 53.5%, respectively. Susceptible rates of MFLX remained 89.4%, 31.3%, 32.9% and 59.5%, respectively. Susceptibilities of CLDM against *F. magna* and *P. asaccharolyticus* were reduced in comparison with 2013 or later. Also, susceptibilities of MFLX against *P. asaccharolyticus* were reduced compared with 2013 or later.

**Conclusion:** Since resistant rates of anti-anaerobic quinolones for *P. anaerobius, F. magna* and *P. asaccharolyticus* have been increasing, continuous surveillance on antimicrobial resistance for anaerobic gram-positive cocci would be needed also in future.

*PI-24*

## ACUTE OSTEOMYELITIS OF THE FEMUR DUE TO *FUSOBACTERIUM NUCLEATUM* IN A 7-YEAR OLD BOY

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**Introduction:** *Fusobacterium* species are anaerobic gram-negative bacilli that are part of the normal flora of the oral cavity, gastrointestinal and genitourinary tracts. The most common invasive infections are pharyngitis, tonsillitis and their complications, such as peritonsillar abscesses and jugular vein thrombophlebitis (Lemierre disease), and infection following bites. We have experienced a rare osteoarticular infection in 7-year old child.

**Case:** A previously healthy 7-year-old boy developed onset of pain in left knee. He visited a private clinic and the radiographs of knee showed normal at the day 18 before admission. He visited department of orthopedic surgery in our hospital with episodes of increased pain at the day 10 before admission. He was well and afebrile, while a magnetic resonance scan showed a cystic lesion in the medial femoral condyle in T1LW/T2LW. After admission, specimens of synovial fluid and bone organization were collected. Also, he received bone curettage with receiving cefazolin. At the 5 day after admission, we have finally diagnosed as osteomyelitis. The bacterial culture revealed *F. nucleatum* as the causative organism. He was treated with 2 weeks of intravenous sulbactam/ampicillin and clindamycin, followed by oral clavulanic acid/amoxicillin. Antimicrobial chemotherapy was guided by clinical response and review of the literature regarding pediatric *Fusobacterium* bone and joint infections. He got a full recovery with no residual pain, limb length discrepancy or growth plate disturbance at 1 year post-surgery.

**Discussion:** According to the previous reports, *Fusobacterium* osteomyelitis in children most commonly occurs in the skull and facial bones, which have been associated with concurrent otitis media, mastoiditis, sinusitis, trauma and dental infection. The cause of our case was considered to be the bacterial translocation from the oral cavity.

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*PI-25*

## *BACTEROIDES FRAGILIS* AND *BACTEROIDES THETAIOTAOMICRON* ON THE PRESENCE OF LACTOFERRIN

Almeida, J.S.S.; Marre, A.T.O.; Boente, R.F.; Teixeira, F.L.; Santos-Filho, J.; Ferreira, E.O.; Domingues, R.M.C.P.; Lobo, L.A.*
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The genus Bacteroides is composed by several species, such as *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*. The prevalence of this species in the human microbiota is regulated by several factors of the host, including proteins with antimicrobial activities such as lactoferrin. This glycoprotein is present in various secretions, and its main activity is to chelate iron ions. When lactoferrin reaches the digestive tract, gastric pepsins digest it generating a bactericidal peptide called lactoferricin. In this context the study was to evaluate growth changes in species *B. thetaiotaomicron* and *B. fragilis*, in response to the presence of holo and apo-lactoferrin, and the peptide lactoferricin B in semi-defined medium for *Bacteroides spp* supplemented with different sources of iron. In addition, we studied the effect of this molecule on the formation of biofilm. The strains used are part of the collection of the Anaerobic Biology lab cultures (IMPPG / UFRJ) and were routinely grown in pre-reduced and anaerobically sterilized (PRAS) culture media. All experiments were performed in an anaerobic chamber (80% $N_2$, 10% $CO_2$ and 10% $H_2$). The strains were cultivated in semi-defined medium without iron for Bacteroides and incubated for 24 hours at 37°C. After this period the cultures were adjusted to $OD_{600}$ = 0,4. The minimal inhibitory concentration (MIC) for holo-lactoferrin, apo-lactoferrin, and lactoferricin B was determined for *B.fragilis* and *B.thetaiotaomicron* in broth microdilution assays. Both strains were resistant to physiological relevant concentrations of lactoferrin (1mg/ml). To evaluate the effect on bacterial metabolism, growth curves were measured spectrometrically ($OD_{600nm}$). Biofilm formation in polystyrene plates was quantified by crystal violet incorporation. No inhibition of growth by lactoferrin was observed, in fact, the effect of lactoferrin was mildly stimulatory for bacterial growth. In contrast, biofilm formation was strongly inhibited in the presence of lactoferrin.

# DETECTION OF BIOFILM PRODUCTION AND ANTI BIOFILM ACTIVITY AGAINST HERBAL EXTRACTS IN INTESTINAL ANAEROBIC BACTERIA—AN '*IN-VITRO*' STUDY

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**Introduction**: Anaerobes exist as the most diversified microbial community in the gastrointestinal tract as normal flora as well as significant pathogen. Biofilm formation is one of the virulence factors which play an important role in their pathogenesis. The present study investigates biofilm formation in various intestinal anaerobes. India being a country with unique culture and heritage, our earlier generation practiced natural herbal medicines for an array of ailments. Hence an attempt was done to demonstrate the antibiofilm activity of herbal extracts used for intestinal infections as well as metronidazole.

**Material & Methods:** A total of 132 Anaerobic bacteria (*B.fragilis group* 49, *C.difficile* 27, *Porphyromonas-Prevotella* group-29, *Peptostreptococci*-23, *Actinomyces*-4) were included in the study. The pure cultures of all anaerobes were grown in BD Gas Pak jar and maintained in Supplemented Brucella agar. Quantitative Biofilm Production Assay was done by Tissue culture plate method. Two fold serial dilutions of Essential oils of *Syzygium aromaticum* (clove), *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger), alcoholic extract of *Garcinia indica* (kokum) & Metronidazole, the drug of choice for anaerobes were analysed to demonstrate the anti biofilm activity. All tests were performed in triplicate.

**Results:** Based on the quantitative biofilm detection majority of the strains of *B. fragilis* group (38), *Porphyromonas-Prevotella* species (23) and *Peptostreptococci* (17) were strongly adherent while *C. difficile* were moderately adherent. Antibiofilm activity revealed that essential oils of clove and nutmeg showed high degree of inhibition while ginger and kokum moderate inhibition. Metronidazole when employed in higher concentrations also prevented biofilm formation .

**Conclusion:** Most of the intestinal anaerobic bacteria exhibited biofilm formation *in vitro*. Tissue culture plate method is suitable to demonstrate biofilm formation in various anaerobes. Medical devices treated with antibiofim agents and high concentration of antibiotics may be developed in the future to overcome this hurdle.

*PI-27*

# COMPARATIVE CHARACTERISTICS OF THE FIRMACUTES AND GRACYLICUTES SULFATE-REDUCING BACTERIA IN THEIR ABILITY TO SYNTHESIZE LECTINS

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Sulfate-reducing bacteria are attracting increasing attention of researchers, since in recent years there have been studies publications in which their presence in patients with ulcerative colitis were noted . Also, there are works that test the ability of sulfate-reducing bacteri to synthesize lectins. Lectins are proteins or glycoproteins structure, not of an immune origin, they are participates in protein-carbohydrate interactions. One of the main properties of lectins is their ability to adhere, which can, apparently, lead to the attachment of cells to the intestinal surface. However, the species diversity of sulfate-reducing bacteria requires a more in-depth study of this issue.

In our experiments, we tested the comparative ability of strains *Desulfovibrio desufuricans* VKM-1799 and *Desulfovibrio vulgaris* VKM-1760 (gracylicutes), as well as *Desulfotomaculum nigrificans* VKM-1492 and *Desulfotomaculum* Z-3 spp. (firmacutes), to synthesize lectins. The growth of sulfate-reducing bacteria was detected by the protein increasing. The activity of lectins was determined by the hemagglutination reaction with erythrocytes of the 1st group of human blood.

The results of the studies showed that all sulfate-reducing bacteria synthesized both endo- and exolectins. However, the activity of exolectins in sulfate-reducing bacteria was much higher than the activity of lectins of the cellular extract. In addition, the titer of activity of the firmacutes bacteria was 2-4 times higher than in the gracylicutes strains. Determination of the dynamics of lectins formation according to cell growth showed that if in the case of gracylicutes bacteria the formation of lectins was observed from the active growth phase of the population, in the case of firmacutes this process was observed at the beginning of the stationary phase of cell growth. An increasing of lactate or pyruvate amounts in the culture medium up to 5 g / l promoted the growth of cells, the titer of lectin activity increased by a factor of 1.5-2.0. However, substitution of these substrates for ethanol led to a sharp decrease in the activity of lectins in all strains studied. A decrease in the titer of lectin activity was also observed when the final electron acceptor of sulfate-reducing bacteria was replaced from sulfates to nitrates and carbonates. The most interesting results were obtained by studying of the temperature effect on the lectins synthesis. Studies have shown that an increase of the temperature to 50°C promoted an increase of the titer of lectin activity by 4.0 times. The optimal pH for the synthesis of lectins was 7.0-7.2.

Thus, it has been established that firmacutes sulfate-reducing bacteria synthesize more active exogenous and endogenous lectins than gracylicutes bacteria.

# THE PRESENCE OF *HELICOBACTER PYLORI* AND *CAMPYLOBACTER UREOLYTICUS* IN THE ORAL CAVITY OF A NORTHERN THAILAND POPULATION THAT EXPERIENCES STOMACH PAIN

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**Objective:** To investigate oral diseases and microbiological conditions, such as the presence of ureolytic bacteria in dental plaque, in relation to experience of stomach pain in a remote adult Asian population.

**Materials and Methods:** Ninety-three adults, 40-60-years old, from the Karen Hill tribe in Northern Thailand with no regular access to dental care were examined. Clinical registrations included number of remaining teeth, caries experience (DFT), plaque index (PI) and periodontal parameters, such as bleeding on probing (BoP), clinical attachment level (CAL), and probing pocket depth (PPD). Interproximal gingival plaque samples were collected and analyzed with the checkerboard (CKB) method for the presence of 14 oral bacterial species.

**Results:** Among the 93 subjects examined, 61 reported daily stomach pain while 32 subjects had no symptoms from the stomach. The subjects with stomach pain had fewer remaining teeth ($p<0.05$), higher caries experience ($p<0.05$) and less BoP ($p<0.01$). Most of the bacterial species were clustered statistically in three factors in a factor analysis, which together explained 65% of the microbiological variance. Factor 1, explaining 43.0% of the variance, was statistically associated with stomach pain ($p< 0.001$). This factor included two gastro-intestinal pathogens *Helicobacter pylori* and *Campylobacter ureolyticus* in addition to four oral bacterial species. There was no association between the amount of calculus, the pH of the plaque, or the urease activity of the plaque, and stomach pain.

**Conclusions:** This study supports previous reports of the oral cavity and the subgingival biofilm, as a reservoir of *H. pylori* and a possible route for re-infection in the stomach. The study also indicates a potential association between *C. ureolyticus*, another ureolytic bacteria, and stomach pain.

*PI-29*

## ASSOCIATION BETWEEN BmoR AND OxyR TRANSCRIPTIONAL REGULATORS AND PHENOTYPICAL CHARACTERISTICS OF *BACTEROIDES FRAGILIS* STRAINS

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*Bacteroides fragilis* is a Gram-negative rod that is found in the gastrointestinal tract as part of the microbiota but can become a pathogen leading to various clinical conditions. This bacterium is the anaerobe most commonly isolated from endogenous infections. Among anaerobes, *B. fragilis* is one of the most resistant to oxygen exposure. *B. fragilis* has a strong response against oxidative stress generated by this exposure, which controls the expression of several genes, such as those encoding detoxifying enzymes. Much of the expression of these genes is controlled by the regulator OxyR that is known to activate a response mainly against $H_2O_2$, but there is another regulator, BmoR, that also acts in this response. Therefore, the aim of this study is to evaluate the prevalence of *bmoR* and *oxyR* in *B. fragilis* strains and their phenotypic characteristics. Initially, the genes *bmoR* and *oxyR* were identified by PCR in a set of *B. fragilis* strains and it was observed that not all strains carried those genes, but the non-identification of these genes in some strains may be due to the presence of polymorphism. These same strains were exposed to atmospheric $O_2$ and $H_2O_2$. In both cases it was observed that some samples were more resistant when compared to the group mean. It was not possible to establish a causal relationship between the presence of those genes and the observed phenotype. Mutant strains of *B. fragilis* were exposed to peritoneal and medullar macrophages, there was no difference in the survival when compared to the wild-type strain, but when we evaluated the ability to form abscesses in C57BL/6 mice it was observed that the absence of *oxyR* led to decreased virulence, suggesting that this regulator may be more involved in the induction of genes related to abscess production. We hope that these study results help to clarify how the BmoR and OxyR regulators act on phenotypic characteristics of *B. fragilis* strains and how these regulators would be related to the ability of *B. fragilis* to prevail in infectious processes.

## COMPARATIVE GENOMICS OF MULTIDRUG-RESISTANT *CAMPYLOBCTER* SPECIES ISOLATES CIRCULATING IN A PRIMATE COLONY

Kim, S.G.;* Sims, L.M.; Summage-West, C.V.; Park, M.S.; Nho, S.W.; Foley, S.L.
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Two *Campylobacter* species, *C. jejuni* and *C. coli*, are major zoonotic pathogens causing gastroenteritis worldwide. A total of 14 *Campylobacter* species from a rhesus monkey colony were isolated, identified, and characterized using multiple biochemical and molecular tools such as automated identification systems (Vitek and BioLog), pulse-field gel electrophoresis, 16S rRNA gene sequences, fatty-acid analysis (MIDI), and protein profile analysis (Biotyper). Of 14 isolates, there were 7 *C. coli*, 6 *C. jejuni* subsp. *jejuni* and 1 *C. fetus* subsp. *vernerealis*, respectively. In addition to intrinsic antimicrobial resistance, 64% (9/14) were ciprofloxacin-resistant and 57% (8/14) were tetracycline-resistant. Whole genome sequence analyses revealed that there were multiple mechanisms for antimicrobial resistance including antibiotic-inactivating enzymes (e.g., β-lactamase), multidrug efflux system (e.g., CmeABC efflux pump), drug target protection protein (e.g., Tet(O)), and alteration of antibiotic targets (e.g., point mutations in the *gyrA* gene). However, there were conflicting observations between genomic and phenotypic traits with regard to microbial resistance. Notably, 33% (3/9) of ciprofloxacin-resistant isolates did not possess *gyrA* modifications that are known to render résistance to the drug. These modifications might not be a major contributing factor in ciprofloxacin resistance.

*PI-31*

## INTERFERENCE OF THE COMMENSAL *BACTEROIDES THETAIOTAOMICRON* IN THE GROWTH AND VIRULENCE OF *ESCHERICHIA COLI* PATOTYPES

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Species belonging to the genus *Bacteroides* are predominant members of the microbiota of the mucous membranes, especially of the intestinal tract of humans and of other animals. One of the most frequently isolated fecal samples is *Bacteroides thetaiotaomicron*, which has been associated with many metabolic functions accessory to the host organism. Besides, new-found research revealed its ability to modulate the expression of a large number of virulence genes of enteric pathogens such as enterohemorrhagic *Escherichia coli* (EHEC). In addition to EHEC there are other categories of pathogens in this species as EPEC (Enteropathogenic *E. coli*) and EAEC (Enteroaggregative *E. coli*), which are associated to diarrheic infections in children up to five years and persistent diarrhea, respectively. The aim of this study was to evaluate the interference of *B. thetaiotaomicron* in the growth and virulence of EPEC and EAEC. The growth of *E. coli* strains will be monitored using a spectrophotometer ($OD_{600}$nm) in modified BHI supplemented with spent medium from *B. thetaiotaomicron*. Agar-motility assay was performed and the presence of spent medium from *B. thetaiotaomicron* decreased EPEC motility while it did not affect EAEC motility. Moreover, the adherence in the assays with Hep-2 cells was affected in EPEC and EAEC exposed to *B. thetaiotaomicron*. The influence in the virulence factors will precede analyzes to characterize specific metabolites to elucidate the interspecies interactions of pathogens and commensals in the intestinal environment which can be involved in the persistence and/or virulent behavior of this species.

Financial support: Capes, CNPq-PIBIC, CNPq, FAPERJ

# PREVALENCE OF DIVISION II (*CFIA*-POSITIVE) ISOLATES AMONG INVASIVE AND NON-INVASIVE *BACTEROIDES FRAGILIS* IN SLOVENIA AS DETERMINED BY MALDI-TOF MS

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Aims of our study were (i) to determine the prevalence of *Bacteroides fragilis* division II (*cfiA*-positive) isolates among invasive and non-invasive isolates from a major Slovenian tertiary-care hospital and (ii) to assess its influence on phenotypic resistance to imipenem.

Consecutive non-duplicate *B. fragilis* isolates from positive blood cultures (invasive) and wound/abdominal cultures (non-invasive) were included in the analysis from 2015 to 2017 period. Data from laboratory information system were matched with mass spectra obtained with Microflex LT instrument and MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany). All mass spectra were reanalyzed using Bruker taxonomy library. Spectra with a log(score) >2.0 were further analyzed with *cfiA* library that separates *B. fragilis*-division I and II isolates based on a log(score) value difference of >0.3. Minimal inhibitory concentrations (MICs) for imipenem were determined with Etest (bioMerieux, Marcy l'Etoile, France), using supplemented Brucella agar and EUCAST breakpoints (S≤2 mg/L, R>8 mg/L). 623 consecutive *B. fragilis* isolates were included in the analysis; 7.5% (n=47) from invasive and 92.5% (n=576) from non-invasive specimens. Altogether, 91.8% (n=572) were division I (*cfiA*-negative) and 8.2% (n=51) division II (*cfiA*-positive) isolates. The proportions of division II isolates among invasive and non-invasive isolates were 14.9% (n=7) and 7.6% (n=576), respectively (p=0.081, ns). In total, 0.8% (n=5) were resistant to imipenem (MIC>8 mg/L); 4.3% (n=2) among invasive and 0.5% (n=3) among non-invasive isolates. All imipenem resistant isolates belonged to division II. Modal MICs (MIC range) were 0.064 mg/L (0.016 mg/L-2 mg/L) and 0.125 mg/L (0.064 mg/L-≥32 mg/L) for division I and II isolates, respectively.

We have successfully screened routine clinical *B. fragilis* isolates for phylogenetic division using MALDI-TOF MS. A large difference in proportion of division II (*cfiA*-positive) isolates between invasive and non-invasive specimens was detected (14.9% vs. 7.6%) in Slovenia during the study period. One double-dilution higher modal MIC was noticed among division II isolates, possibly indicating a low-level expression of carbapenemase.

*PI-33*

## *CITRONIELLA SACCHAVORANS* GEN. NOV. SP. NOV. ISOLATED FROM THE GASTROINTESTINAL TRACT OF AN INDIGENOUS PERUVIAN COMMUNITY

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The family *Peptoniphilaceae* consists of nine validly published genera (*Anaerococcus*, *Anaerosphaera*, *Ezakiella*, *Finegoldia*, *Gallicola*, *Helcococcus*, *Murdochiella*, *Parvimonas* and *Peptoniphilus*). Gram-positive anaerobic cocci are members of the commensal flora of animal and human gastrointestinal tracts but are also recovered from human clinical samples. Strain M6.X9$^T$ was isolated from a fecal sample obtained from one member of a traditional coastal Peruvian community. A focus of our group was to use data from 16S rRNA molecular inventories as road maps to target previously uncultivated groups to investigate phylogenetic, physiological, biochemical, and chemotaxonomic properties. Using a polyphasic taxonomic approach, it is proposed that strain M6.X9$^T$ represents a novel genus and species within the family *Peptoniphilaceae*.

Freshly voided fecal samples were collected from members of the Afro-Peruvian community of Cruz Verde in Tambo de Mora, region Ica, in Peru. Multiple enrichments were prepared, and isolates were sub-cultured to purity on blood agar and then screened using 16S rRNA analysis gene sequence analysis. Strain M6.X9$^T$ is a Gram-positive staining, non-motile, non-sporeforming coccus-shaped obligately anaerobic bacterium. Fermentation end products from PYG were determined to be acetate and methyl-succinate. The diamino acid isomer present in the cell wall was Lysine. An interesting characteristic of this isolate is its ability to ferment sugars, a feature that is not common with species of other genera in this family.

Our investigations demonstrate that remote indigenous communities harbor novel microbial taxa and that human gut microbiome studies should employ culture-based approaches to augment molecular investigations. It's only by isolating and characterizing the microbes from these environments, can we truly study their function. Based on the phenotypic, chemotaxonomic, and phylogenetic results, the organism is a member of a novel genus for which the name *Citroniella sacchavorans* gen. nov. sp. nov., is now proposed. The isolate is named after Diane Citron, for her many contributions to clinical microbiology.

# EFFECT OF THE HUMAN GUT METABOLOME ON *VIBRIO CHOLERAE* VIRULENCE GENE EXPRESSION

Pauer, H.;*[1] Teixeira, F.L.;[2] Ferreira, R.B.R.;[2] Allen-Vercoe, E.;[3] Lobo, L.A.;[2] Domingues, R.M.C.P.;[2] Antunes, L.C.M.[1]
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Humans live in symbiosis with a diverse community of microorganisms, which has evolved to carry out many specific tasks that benefit the host. Within the chemical diversity of the gastrointestinal tract, many of the molecules found could constitute chemical cues for the communication between the microbiota and invading pathogens. The goal of this work was to investigate the production of molecules by the human gut microbiota that possess biological activity against the human pathogen *Vibrio cholerae*. In order to probe the unknown properties of the human gut metabolome, we extracted molecules from fresh feces of a healthy donor, from *Clostridium citroniae*, and *Bacteroides thetaiotaomicron*, and tested the effect of the extracts on *V. cholerae*, comparing bacterial growth in the absence and presence of the small-molecule extract. In these experiments, we observed that molecules present in the gut metabolome as well as those produced by *C. citroniae* inhibit *V. cholerae* growth, although the exact reason for this effect is unknown. The extract from *B. thetaiotaomicron* did not affect pathogen growth. The next step was to determine if the molecules involved could modulate the expression of virulence-related genes. Expression analyses revealed that small molecules from the fecal extract as well as the extract from *C. citroniae* significantly altered the expression of at least four genes related to *V. cholerae* virulence (*ctxA, ctxB, zot, tcp*). RNA sequencing was then performed to investigate the effect of the fecal extract on global gene expression profiles of *V. cholerae*. Our results show that some small molecules present in the gut metabolome have a significant impact on the microbe-microbe interactions established in this environment and provide a framework for the study of other small molecules involved in microbiota-pathogen interactions. Future work will likely reveal new molecules in the intestinal environment that are involved in interspecies interactions. Many of these may have potential antivirulence activity that can be pursued for therapeutic purposes.

Financial support: CNPq, CAPES, FAPERJ

*PI-35*

## GENOMIC DIVERSITY OF HUMAN GUT-ASSOCIATED TREPONEMA INFERRED FROM SHOTGUN METAGENOMIC DATASETS

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Shifts in human subsistence lifestyles, specifically the adoption of industrial agricultural practices has resulted in changes to the composition and diversity of the human gut microbiota. In addition to a decrease in overall microbial richness, these changes also include reduced prevalence and even complete absence of certain microbial taxa among industrialized populations. It is essential to understand the contribution of these "missing microbes" to overall gut function and to evaluate the impact of their loss on human health. Ideally, this would require isolation of these microbes in pure culture followed by functional characterization. However, challenges in obtaining viable samples (often collected from remote geographical locations), coupled with lack of information on nutrient requirements and growth conditions for several of these taxa, severely impact isolation based approaches. In contrast, advances in high-throughput sequencing and bioinformatics algorithms (genome assembly, binning, and annotation), now allow for reconstruction of partial and near-complete genomes from complex metagenomic datasets. In this study, we generated taxonomic inventories from published shotgun metagenomic datasets for over 3300 individuals (14 populations) and screened for the presence of Treponema. This analysis revealed the presence of multiple Treponema phylotypes among non-industrial populations. Shotgun metagenome datasets from individuals sharing specific phylotypes were merged and assembled using Ray-meta, followed by contig binning using MetaBAT. Treponema specific bins were identified using protein blast against the non-redundant protein sequence database from NCBI. A core set of marker proteins conserved across Spirochaetes was recovered for each reconstructed Treponema genome and used to visualize the biogeographic distribution of these strains across human populations.

***1300*** ***SESSION I:*** **ANAEROBIC METHODOLOGY**



Posters will be presented in Poster Session I
Wednesday, July 11 1300-1400.

*PI-36*

## EVALUATION OF THE SENSITITRE ANAEROBE MIC PLATE COMPARED TO ROUTINE ANTIMICROBIAL SUSCEPTIBILITY TESTING BY ETEST OF *BACTEROIDES FRAGILIS* GROUP ISOLATES

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**Introduction:** Agar dilution is the reference standard for antimicrobial susceptibility testing (AST) of anaerobes, a method few laboratories have the resources to perform. Due to ease-of-use, most labs have implemented Etest for AST. The purpose of this study was to compare the TREK Sensititre anaerobe panel to standard of care Etest results for AST of *Bacteroides fragilis* group.

**Method:** Thirty *Bacteroides fragilis* group isolates were tested by Etest (bioMérieux) and the Sensititre Anaerobe MIC Plate (AN02B, TREK Diagnostic Systems; includes 15 antimicrobial agents) to determine minimum inhibitory concentrations (MIC). Only one isolate per patient was included in this study. The antimicrobials evaluated included: metronidazole (MET), amoxicillin-clavulanate (A/C), clindamycin (CLI), meropenem (MER), and tetracycline (TET). AST was performed and interpreted following CLSI guidelines. Hands-on-time, categorical agreement (CA) and essential agreement (EA) were evaluated.

**Results:** All isolates were susceptible (100%) to A/C, MET and MER. For CLI and TET, 73% and 23% were susceptible, 0% and 10% were intermediate, and 27% and 67% were resistant, respectively. The CA between methods for all 5 antimicrobials tested was 100%. No minor, major or very major errors were observed. The EA was variable and was found to be 97% for A/C and TET, 93% for MER, 69% for CLI and 50% for MET. When EA was not achieved, the Sensititre panel generated higher MIC values than the Etest method. The hands-on-time for setup of Sensititre panels was ~4 min compared to ~5 min for Etest and reading took ~2 min compared to ~1 min for Etest.

**Conclusion:** The Sensititre performed comparably to Etest for determining AST of clinically significant *B. fragilis* isolates achieving a CA of 100% for all antimicrobials evaluated. Sensititre plates provide results for more antimicrobial agents in a condensed and easy to use panel that takes slightly less time to setup.

# *CLOSTRIDIOIDES DIFFICILE* STRAIN CHARACTERIZATION AND DEVELOPMENT OF AN ALTERNATIVE MALDI-TOF MS TYPING METHOD

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*Clostridioides difficile* is an opportunistic and nosocomial pathogen, responsible for developing the CDI (*Clostridioides difficile* infection) by producing potent toxins (TcdA, TcdB and binary toxin). CDI can cause a simple diarrhea or progress to pseudomembranous colitis, a severe a colonic inflammation. Recently, the epidemiology of *C. difficile* has dramatically changed with the appearance of an epidemic strain, the NAP1/BI/027. Until today, there is no notification of the 027 ribotype in Brazil, but two ribotypes were exclusively isolated in the country, 133 and 135. PCR-ribotyping and PCR-multiplex are the main methods performed worldwide to ribotype and detect virulence genes, respectively. In Latin American countries, the PCR-Ribotyping is not performed. Hence, the aim of this study was to develop and validate an in-house MALDI-TOF MS database for *C. difficile* typing by using a fast protein extraction procedure. Besides, a complete screening for the characterization strains used in the study (33) was performed. To create the database, spectra were generated with 19 ribotypes following by BioNumerics analysis. PCR-Multiplex showed that 34% of strains were non-toxigenic and 64% toxigenic. The biofilm production was also evaluated and showed that 88% of the strains were strong biofilm producers, while 12% were moderate producers. All strains were susceptible to metronidazole and vancomycin and 33% displayed resistance to clindamycin. The cytotoxic assay demonstrated that 81% of the strains were toxigenic (titration ≤1:8). A motility test categorized the strains with high motility (42.4%), moderate (15.2%) and low motility (42.4%). The MALDI-TOF typing has been successfully validated and implemented, with discriminating rates of 89.5% (17/19 ribotypes), representing a reliable and faster alternative for typing *C. difficile* strains. Altogether, the characterization of our culture collection can gather epidemiological data to the country and contribute for a better elucidation of the virulence of strains involved in CDI in Brazil.

Financial support: CNPq, FAPERJ, CAPES.

*PI-38*

# SEQUENCING / METHODS EVALUATION OF COMMONLY USED PRIMERS TARGETING 16S rRNA GENES IN NEXT-GENERATION SEQUENCING (NGS) BASED STUDIES

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Next-generation sequencing (NGS) technologies became an important tool for studying the microbial etiology, ecology, and biodiversity of mixed anaerobic infections. Sequencing of hypervariable domains of 16S rRNA genes is a well-established and effective approach to investigate complex microbial communities in the sample of interest. Several variable regions of the 16S rRNA gene (V1-V9) can be targeted for sequencing using specific (containing only G, A, T, C) or wobbled (plus W, S, M, K, R, Y, B, D, H, V or even N) primers for the region of interest. However, for the most applied Illumina short read format a choice of region (most prominent are V123 and V45) and primer has to be made. Besides the DNA extraction method, the primer choice is an essential key factor that may alter the NGS experiment results.

Here, after performing a meta-analysis of NGS-literature we aim to review the PCR-primer sequences applied and the resulting oral microbial diversity along with a comparison of their advantages and limitations. Our preliminary results show significant differences in the detection of oral microbial taxa—including many anaerobes—related to the choice of primer pair. However, further studies are needed and the technology has to be improved to allow longer reads before optimal primers and sequencing protocols for oral microbiome analysis can be established.

# COMPARISON OF VITEK MS SYSTEMS AND VITEK 2 COMPACT ANC CARD FOR IDENTIFICATION OF ANAEROBIC BACTERIA

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**Background:** Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is high-throughput, rapid and inexpensive technique that gives significant improvement for identification of microorganisms. VITEK MS plus is a commercially available MALDI-TOF MS systems provided by bioMerieux. The VITEK 2 compact anaerobe and Corynebacterium (ANC) identification card, which is also commercially available from bioMerieux, is based on conventional biochemical method for identification of corynebacteria and anaerobic species. The reports that compare the performances of those two methods are rare. The aim of this study was to evaluate the performance of the MALDI-TOF MS and ANC card for identification of anaerobes.

**Methods:** A total of 170 clinically isolated anaerobes comprising 12 different genera and 26 species were identified by the VITEK MS plus with version 3.0 database and ANC card with VITEK 2 identification system with version 07.01, respectively. 16S rRNA gene sequencing was used as reference method for accuracy in the identification.

**Results & Discussion:** VITEK MS plus gave high confidence identification results for gram-negative anaerobes. All of the tested strains were correctly identified. On the other hand, 13% of Genus Bacteroides strains were misidentified in VITEK 2 compact ANC card. It is suggested that, VITEK MS plus is rapid, easier and more cost-effective method compared to the VITEK 2 ANC card. The databases currently available for VITEK 2 compact ANC card should be updated to enhance performance.

*PI-40*

## DEVELOPMENT OF AN IMMUNOASSAY FOR THE RAPID DETECTION OF *CLOSTRIDIUM SEPTICUM* ALPHA TOXIN IN HUMAN SERUM AND PLASMA

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**Purpose:** The ability to rapidly diagnose *C. septicum* infection in immunocompromised patients would benefit greatly from the development of a robust, clinically validated immunoassay.

**Methods and Results:** Using a modification of the protocol of Lancto *et al.*, (Avian Diseases 58:566-571, 2014), a non-cytolytic synthetic construct of the *C. septicum* alpha toxin with the internal virulence factor domain (aa204-231) removed was cloned and expressed in *E. coli*. The sequence of the resultant protein was confirmed by mass spectroscopy. Three BALB/c mice were immunized with the protein in complete Freund's adjuvant and boosted twice using the toxin in incomplete Freund's adjuvant. The spleens of the mice were used to produce hybridomas, and the cell supernatants screened for their ability to bind the non-cytolytic alpha toxin. A pair of monoclonal antibodies were chosen that recognized distinct epitopes on the alpha toxin, and these were used to develop and ELISA. The ability of the ELISA to detect both the non-cytolytic alpha toxin as well as native alpha toxin in human serum and plasma was tested.

**Conclusions:** It is possible to produce an ELISA capable of detecting the alpha toxin of *C. septicum*. Whether this assay, or a modification of it, can be used in a clinical setting will require additional testing. Using a similar technique, it should be possible to generate immunoassays to toxins from other Clostridium species.

# HIGH-THROUGHPUT SCREENING FOR VIABLE BACTERIA IN FECAL MATERIAL USING PROPIDIUM MONOAZIDE

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Advances in molecular methods and high throughput sequencing, have allowed for rapid characterization of microbial communities, specifically through taxonomic inventories obtained via amplification and sequencing of hypervariable regions in the 16S rRNA gene. These inventories serve as molecular road maps and are critical to our understanding of microbial community characteristics including richness, evenness, and taxon abundance, and enable the identification of key microbial taxa within an ecology. Once identified, it is essential to isolate these organisms in pure culture in order to evaluate specific hypotheses on their functional role within the community.

Successful recovery of microorganisms from a community require accurate media formulations, growth conditions, and the presence of live cells within the sample. The viability of microorganisms within a sample are dependent upon several factors including collection method, environment, storage conditions, and biological properties of the organism (ability to form spores). Current approaches to generating taxonomic inventories rely on total DNA preserved in a sample and contain contributions from both live and dead cells.

In this study, we integrate Propidium monoazide (PMA) treatment of fecal samples, with high throughput 16S rRNA gene sequencing, to identify samples containing viable cells from microbial taxa associated with the rural human gut ecology. Fecal samples (n=20) from a hunter-gatherer population were made into a slurry and DNA was extracted from two aliquots (with and without PMA treatment). The V4 hypervariable region of the 16S rRNA gene was amplified using barcoded PCR primers, and resulting amplicons pooled and sequenced on an Illumina MiSeq instrument (v2 chemistry, 2 x 250bp). Reads were quality filtered, trimmed, and merged using 'pear,' and assigned to Operational Taxonomic Units (OTUs, 97% similarity), using the UPARSE pipeline, and resulting 'biom' file rarefied to a depth of 10,000 reads per sample. Overall, PMA treatment resulted in a decrease in community evenness ($p < 0.05$), while richness metrics showed no significant difference. Several microbial genera associated with the rural gut ecology including *Prevotella* ($p < 0.03$) and *Catenibacterium* ($p < 0.01$) showed increased proportions in the viable fraction, while other taxa such as the fastidious anaerobe *Treponema* showed a decrease. Thus, this method could serve as a diagnostic tool to identify appropriate samples for targeted isolation of microbial taxa and improve cultivation success.

*PI-42*

## FUSOPORTAL: AN ONLINE DATABASE OF HYBRID MINION SEQUENCED, ASSEMBLED, AND FUNCTIONALLY ANNOTATED *FUSOBACTERIUM* GENOMES

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Here we present FusoPortal, an online database of complete *Fusobacterium* genomes that were sequenced using hybrid MinION long-read sequencing, and assembled and annotated using a diverse portfolio of open-source software. This resource provides the first fully assembled genomes for several strains of virulent *Fusobacterium nucleatum*, many of which are associated with the development of colorectal cancer. FusoPortal has been initiated with eight complete genomes, of which 7 were previously only drafts that varied from 6-200 contigs. Significant efforts were made to provide data in easily downloadable formats, fostering a powerful and efficient experience for users. We further showcase that FusoPortal is superior for virulence factor identification, and have corrected a significant number of Type 5 secreted autotransporters and MORN2 domain containing proteins that are misannotated in UniProt. In summary, FusoPortal is the first database of MinION sequenced *Fusobacterium* genomes, and this powerful resource will be expanded in the near future to include >25 genomes to aid the scientific community.

# COMPARISON OF BROTH MEDIA FOR RECOVERY OF *CLOSTRIDIUM DIFFICILE* FROM ENVIRONMENTAL SAMPLES

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An evaluation of 3 selective broth media was conducted to determine the most rapid and sensitive media for detection of viable *Clostridium difficile* spores from flocked swabs used to sample primarily the healthcare environment but also possibly the human microbiota. Three broth media: Cycloserine cefoxitin fructose broth (CCFB), *C. difficile* Banana Broth (BB), and Cycloserine cefoxitin mannitol broth with taurocholic acid lysozyme and cysteine (CCMB-TAL) were compared by inoculating with 3 *C. difficile* spore levels ($10^4$, $10^2$, and $10^1$ spores), at varying inoculum volumes (10µL, 100µL, and 1mL). Inoculation was done either by direct addition of the spore suspension into the broth or spiking of a swab (10µL or 100µL only) which was then immediately placed into the broth. Broth tubes (n=5 per sample type) were incubated anaerobically (CCFB and CCMB-TAL) or aerobically (BB) at 36°C and observed for growth at 24, 48, and 72h.

When spores were directly inoculated into the broths, more CCFB tubes produced growth after 24h (80% and 100% of tubes with 10 and 100µL of $10^1$ spores, respectively) than CCMB-TAL or BB tubes. If 1mL suspension was added to the CCFB tube, the number of positive tubes declined to 20%. After 48h, 97% of CCFB and 100% of BB tubes at all inoculum levels and volumes were positive, while 82% of CCMB-TAL were positive. CCMB-TAL required $10^2$ spores to be 100% positive at 48h. After 72h, 98% of CCFB and 100% of both BB and CCMB-TAL were positive.

After 24h, all broths containing swabs spiked with $10^1$ spores were negative, and 20% of CCFB tubes and 50% of BB tubes were positive if spiked with $10^2$ spores. After 48h, all broth tubes at the $10^1$ spiked swab level were positive.

At 24h, growth was observed earlier for CCFB than BB or CCMB-TAL for the direct inoculation method; BB produced more positive tubes for the swab inoculum method. These results indicate that BB was more sensitive for growth of *C. difficile* on environmental swabs; CCFB was more sensitive for direct spore inoculation only. Further work will study the impact of background organic material and other organisms on the detection of *C. difficile* spores from environmental samples.

*PI-44*

## SUSCEPTIBILITY TESTING OF RIFAMPIN FOR *CUTIBACTERIUM* SPP.: IS IT TIME TO ESTABLISH A BREAKPOINT?

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Long acknowledged to be skin commensals and surface contaminants, *Cutibacterium acnes* and related genera and species are increasingly recognized as pathogens in device-associated infections. Rifampin has been used to treat such joint infections due to its anti-biofilm activity. However, Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints and anaerobic quality control (QC) ranges for *Cutibacterium* against rifampin have not yet been pursued. Therefore, we sought to assess the minimal inhibitory concentration (MIC) distributions of rifampin against 83 clinical *Cutibacterium* isolates, comparing agar dilution (AD) to Etest (bioMérieux). 35 orthopedic isolates from Mayo and 48 clinical isolates from Weill Cornell were subcultured from frozen stock. 80 isolates were *C. acnes*; the remainder were other *Cutibacterium* spp. AD was performed at Mayo Clinic on all isolates according to CLSI methods using Brucella agar supplemented with hemin (5 mg/mL), vitamin K1, and laked sheep blood (range: 0.03 – 256 mg/mL) with 0.5 McFarland inocula. Etest was performed at both institutions (range: 0.002- 32 mg/mL) using Brucella blood agar plates with 1.0 McFarland inocula. Plates were incubated anaerobically at 35°C for 42-48 hours prior to reading endpoints. QC organisms were *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium difficile* ATCC 25285. By AD, MIC values for all isolates measured at ≤0.03 mg/mL. Etest MIC values ranged from ≤0.002 – 0.004 mg/mL. MICs of QC *B. fragilis* were 0.032 to 0.64 mg/mL, *B. thetaiotaomicron* 0.125 (Etest) and 0.12 (AD) mg/mL, and *C. difficile* ≤0.002 (Etest) and ≤0.03 (AD) mg/mL. Overall, rifampin MICs for isolates and QC organisms were low (≤0.002 to 0.125 mg/mL). Given rifampin's increasing clinical use for treatment of *Cutibacterium* orthopedic infections and the low measured MICs, CLSI should consider establishing QC ranges for anaerobic testing of rifampin and develop *Cutibacterium* epidemiological cutoff values (ECVs) for rifampin.

***1300*** ***POSTER SESSION I:*** **ANAEROBIC PATHOGENESIS**



Posters will be presented in Poster Session I
Wednesday, July 11 1300-1400.

*PI-45*

# CHARACTERIZATION OF ANAEROBIC AND MICROAEROPHILIC BACTERIA ISOLATED FROM ORODENTAL INFECTIONS WITH AN EMPHASIS ON *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* (A.a) FROM COASTAL KARNATAKA, S. INDIA & GENOTYPING OF A.a BY ARBITRARILY PRIMED–PCR

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**Introduction:** The role of Anaerobic bacteria and *A. actinomycetemcomitans* in the etiology of Orodental infections has been well documented. However not many reports are available from the Indian subcontinent. The present study was undertaken to denote the prevalence of anaerobes and *A.a* in various orodental infections encountered in and around coastal Karnataka region.

**Objectives:** To investigate the etiological agents from orodental infections with an emphasis on *A.a* and to perform MALDI TOF analysis, detection of JP2 clone if any and genotyping by Arbitrarily Primed PCR (AP-PCR) for the randomly selected, isolates of A.a.

**Methods:** Paper points, gingival biopsy or sub gingival plaques were collected from 976 cases of orodental infections and 240 age matched control group in Reduced Transport Fluid (RTF) and inoculated in respective culture media to obtain a semiquantitation. Anaerobic and microaerophilic conditions were provided employing BD Gaspak system. Colonies were identified using biochemical characterization. Randomly selected 105 strains of *A.a* were subjected to MALDI TOF analysis ((Biomerieux), conventional PCR to rule out JP2 clone & Genotyping by AP-PCR.

**Results:** A total of 1357 pathogens were obtained from 976 cases. Predominant isolates were *Porphyromonas-Prevotella* group (421) followed by *A.actinomycetemcomitans* (272), *Peptostreptococcus anaerobius* (255), *Fusobacterium nucleatum* (238) *Tannerella forsythia* (65) and *Streptococcus mutans* (106) . 105strains of *A.a* subjected to MALDI TOF MS analysis were the best matched with 6 reference *A.a* strains from the data base. None of these 105 had JP2 clone. 97 strains could be genotyped among the 105 by AP-PCR and 8 strains were nontypable. The hierarchal cluster analysis was done using Euclidean distance method which distinguished a total of 19 clusters or genotypes.

**Conclusions**: Anaerobes and *A.a* were isolated in significant numbers in this study. If facility is available, implementation of MALDI TOF as the first step for identification will shorten the turn around time which in turn will improve the patient care . As reported in other Asian countries, JP2 clone was not reported in our study also. Genotyping by AP- PCR will be helpful in the epidemiological point of view.

## CONSERVATION OF A NOVEL SPORE GERMINATION PATHWAY IN *PARACLOSTRIDIUM BIFERMENTANS*

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In endospore-forming organisms, the vegetative cells form dipicolonic acid (DPA)-rich, metabolically dormant spores upon nutrient or environmental stressors. These spores identify environmental signals (germinants) using proteins in the spore (germinant receptors) to return to the vegetative state through a process called germination. Germination is an attractive target for novel therapeutics for pathogenic clostridia like *Clostridiodes difficile*. Previous research in the field of germination of bacterial spores has been conducted on the model spore-former, *Bacillus subtilis*. Recent research on germination in *C. difficile* has shown that there are significant differences in the germinant receptors used to initiate the germination process and differences in the order of events that occur post-germinant recognition. *B. subtilis*, and the majority of other well-studied spore forming bacteria, encode the canonical *ger*-type germinant receptors and releases DPA before cortex is degraded. However, *C. difficile* encodes a non-canonical germinant receptor (*csp*-type) and initiates cortex degradation before releasing the DPA from the spore core. We hypothesized that other organisms may germinate similarly to the mechanism of spore germination observed in *C. difficile*. Here, we examined another clostridial species, *Paraclostridium bifermentans*, to test the hypothesis that the mechanism of germination observed in *C. difficile* can be observed in another clostridial species. *P. bifermentans* encodes homologs of the non-canonical *csp*-type germinant receptors. In *P. bifermentans*, we show that cortex degradation is required before DPA can be released from the spore core. Additionally, we demonstrate that release of DPA is mechanically gated in response to cortex degradation. These results suggest that the mechanism of germination observed in *C. difficile* is found in other organisms and that the 'outside-in' mechanism of spore germination is a novel spore germination pathway.

*PI-47*

## DETECTION OF *cfxA*/*cfxA2* GENES FOR CLINICAL ISOLATES OF *PREVOTELLA* SPECIES IN JAPANESE UNIVERSITY HOSPITAL

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**Background:** *Prevotella* species are representative bacteria responsible for various anaerobic bacterial infections. Although β-lactam antibiotics are often prescribed to treat these infections, some reports have shown that *Prevotella* species have developed resistance to β-lactam antibiotics because of the production of β-lactamases. The *cfxA/cfxA2* genes are considered as responsible gene to production of β-lactamases. However, the possession rates of these genes for *Prevotella* species have not revealed so far in Japan. We investigated the presence of *cfxA/cfxA2* genes for *Prevotella* species.

**Materials and Methods:** We extracted DNA from 204 of *Prevotella* species (98 strains of *P. bivia*, 63 strains of *P. intermedia*, 43 strains of *P. melaninogenica*) isolates at Aichi Medical University Hospital from 2010 to 2017. Isolates were screened for the presence of *cfxA/cfxA2* genes using Polymerase Chain Reaction (PCR). The antimicrobial activity was examined by RAISUS automated method (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan).

**Results:** The *cfxA/cfxA2* genes for *Prevotella* species were detected at the rate of 68.6% (140/204), 82.7% (81/98) for *P. bivia*, 61.9% (39/63) for *P. intermedia*, 46.5% (20/43) for *P. melaninogenica*. In consideration with antimicrobial susceptibility and resistant genes, 83.6% and 17.9% in *Prevotella* species, 86.4% and 28.4% in *P. bivia*, 89.7% and 5.1% in *P. intermedia*, 60.0% and 0% in *P. melaninogenica* possessing resistance genes were resistant to ampicillin and amoxicillin/clavulanate, respectively.

**Conclusions:** This study revealed high prevalence of the *cfxA/cfxA2* genes in *Prevotella* species. We would need continuous surveillance for antimicrobial resistance including responsible resistant genes not only for *Bacteroides* species but for *Prevotella* species.

# MOLECULAR BASES OF *ACHOLEPLASMA LAIDLAWII* ADAPTATION TO ENVIRONMENTAL CONDITIONS

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Many bacteria of the group Mollicutes are parasites of the higher eukaryotes, some cause serious infections, and the main contaminants of cell cultures and vaccine preparations. The rapid development of the omics technologies resulted in the increased number of genome sequences and the transcriptome and proteome studies of mollicutes. However the molecular bases of adaptation of these bacteria to various environmental factors determining their survival in nature, formation of the "parasite-host" system *in vivo* and *in vitro*, and realization of virulence, remain elusive. *Acholeplasma laidlawii* (the ubiquitous mollicute) showing a high level of resistance to stressors is a well-suited model to study adapting mollicutes to environmental conditions.

The analysis of genomic profiles and vesicular proteomes of *A. laidlawii* cells grown in optimal and stressful conditions was the object of our work.

Classical microbiological methods as well as omics profiling including Illumina dye sequencing and 1D-LC-ESI-MS/MS were used for analysis of the *A. laidlawii*.

Adaptation to low temperature and substrate limitation in *A. laidlawii* is accompanied by multiple changes in genomic profile and proteome of bacterial vesicles associated with many genes and proteins involved in the fundamental cellular processes, including virulence factors of microorganisms. The revealed differences of proteins determining bacterial virulence at adapting *A.laidlawii* to different stressors may indicate a differential pathogenic potential of the infect. The differences of mutational changes and modulation of vesicular proteomes in the *A. laidlawii* cells grown under optimal and stressful conditions found in the study suggests that responses to stressors may be associated with the considerable rearrangements of biochemical processes of the bacterium and different strategies for the reactivity of cells to various types of stressors.

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*PI-49*

## A STUDY OF THE TARGET ANAEROBES ASSOCIATED WITH PERIODONTAL INFECTIONS IN KUWAIT

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Periodontal diseases are a group of chronic infections that destroy tissues surrounding and supporting the teeth. The objective of this was to investigate the target anaerobes associated with periodontal diseases in Kuwait. Patient with chronic periodontitis, seen at the Kuwait University Dental Clinics, were recruited into this study during a period of 15 months. Samples were collected using Gutta Percha Points directly from inside the gingival pockets and transported in sterile Ringer's solution and pre-reduced thioglycolate broth to the Laboratory. One set was cultured on appropriate media and the other was used for PCR assays. DNA sequencing and semi-quantitative PCR were performed for *Prevotella* spp. only. A total of 30 patients stratified according to the severity of the disease; mild (3 patients), moderate (14), and severe (13), were studied. Thirty-one healthy individuals were included as controls. Thirty percent of the patients were between 50-59 years' age group. Female-to-male ratio was 1:2. The ratio of Kuwaitis-to-non-Kuwaitis was 1:3.3. *Aggregatibacter actinomycetemcomitans* were found in 23.3% versus 16.1% of cases and controls, respectively. All the 30 patients harbored *Fusobacterium* spp. compared to 93.5% of the controls. *Porphyromonas gingivalis* was detected in 33.3% of patients versus 6.4% of the controls ($P<0.0001$). *Tannerella forsythia* was detected in 83.3% versus 51.6% patients and controls, respectively, ($P<0.0001$). *Parvimonas micra* was detected in 90% versus 51.6%, respectively ($P<0.0001$) and *Treponema denticola* was detected in 70% versus 29%, respectively ($P< 0.0001$). *Prevotella* spp. were detected in all the patients and controls; the majority was *P. nigrescens* detected in 70% and 100% of patients and controls, respectively. There was no significant difference in DNA concentrations of *Prevotella* spp. between patients and controls by semi-quantitative PCR assay. In conclusion, all the target anaerobes, except *Prevotella* spp. and *Fusobacterium* spp., were significantly associated with periodontitis in our study.

# *CAMPYLOBACTER RECTUS* CIAB CONTRIBUTES TO HOST CELL SPECIFIC INVASION AND MAY BE AN ANTI-INFLAMMATORY EFFECTOR

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To investigate potential pathogenesis-related genes (conserved secretion systems) for *Campylobacter rectus* (a human oral anaerobe), a comparative genomics approach has been used based on *Campylobacter jejuni* (a microaerophile) and human gastrointestinal pathogen. *Campylobacter rectus* has been linked to periodontitis, isolated from Barrett's esophagus, appendicitis, and oral and extra-oral abscesses, and has also been implicated in bacteremia. In recent years, *C. rectus* has been associated with pre-term births and low birth weight thus qualifying as an emerging pathogen.

**Methods and Results:** Molecular tools for mutagenesis and real-time PCR (reference genes), as well as adherence and invasion assay adaptation (from microaerophilic to anaerobic) have been developed to focus on the campylobacter invasion antigen B (*ciaB*), a flagellar/T3SS secretion system effector, as a prelude to further studies of pathogenesis related pathways involving conserved secretion systems. In particular, using optimized adherence and invasion assays (anaerobic), *C. rectus* ciaB was shown to be non-essential for adherence, but required for optimal invasion of BeWo (placental) cells. Additionally, *C. rectus* appeared to target specific cells (BeWo vs. gingival fibroblast cells) thus providing further evidence for its potential role in adverse pregnancy outcomes by differential invasion of placental cells. Preliminary host response studies with BeWo cells (real-time PCR gene array) suggest that CiaB plays an anti-inflammatory role that may facilitate immune response evasion.

Molecular and cellular assays have been established to examine secretion system mutants in *C. rectus* and thus elucidate conserved or novel pathogenesis pathways.


JULY 9-12, 2018
ANAEROBE 2018
Las Vegas, Nevada

***1300*** ***POSTER SESSION II: CLOSTRIDIUM* SPP.**



Posters will be presented in Poster Session II
Thursday, July 12 1300-1400.

*PII-1*

## COMPARISON OF FECAL CLOSTRIDIUM PERFRINGENS STRAINS OF PATIENTS WITH AUTISM SPECTRUM DISORDERS WITH STRAINS FROM HEALTHY AND OBESE PERSONS

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Autism affects about 1% of the population. Autism spectrum disorders (ASD) is a range of conditions characterized by persistent deficit in social interactions. ASD patients often suffer from gastrointestinal disorders. The intestinal microbiota of autistic patients significantly differs from that in healthy individuals. The aim of the study was to compare the profile of toxins produced by fecal *C. perfringens* strains of children with ASD, with isolates of healthy individuals and obese subjects. This study included 111 strains of C. perfringens: 49 isolates from 29 children with ASD, 30 - from 17 healthy individuals and 32 - from 24 young obese subjects. Strains were identified by VITEK 2 Compact (bioMérieux, Marcy L'Etoile, France). Alpha, beta, beta2, epsilon, iota and enterotoxin genes in *C. perfringens* strains were detected using appropriate PCRs. The alpha toxin gene (*cpa*) was present in all 111 examined strains (100%). The beta2 gene (*cpb2*) was detected in 45/49 strains (91.8%) isolated from children with ASD, 17/30 (56.7%) isolates from healthy subjects, and 12 of 32 (37.5%) isolates from obese subjects. C. perfringens strains with *cpb2* gene were detected in 27/29 ASD patients (93.1%), 10/17 healthy subjects (58.8%) and 11/24 (45.8%) obese subjects. Beta2 toxin encoding *cpb2* gene was significantly more common in strains isolated from ASD patients.

Further research to explain observed phenomena and pathophysiology of beta2 toxin is required.

# EXPLORING THE POTENTIAL THERAUPETIC AGENT MEPACRINE AGAINST *CLOSTRIDIUM PERFRINGENS* ENTEROTOXIN *IN VIVO*

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*Clostridium perfringens* enterotoxin (CPE) is a pore-forming toxin that causes diarrhea associated with bacterial food poisoning and antibiotic-associated diarrhea when humans are infected with CPE-positive *C. perfringens* type A strains. Some cases of CPE-associated disease are fatal, so the development of therapeutic tools for these infections is needed. In a recent study, mepacrine, an antiprotozoal drug, was found to reduce CPE-induced cytotoxicity in enterocyte-like Caco-2 cells *in vitro*. This protection did not involve inactivation of CPE, but a reduction of CPE-induced pore formation in Caco-2 cell membranes. In the present study, we tested two animal models to evaluate a potential role of mepacrine in preventing intestinal disease and enterotoxemia. First, ligated small intestinal loops were prepared in Balb/c mice and challenged with 50 µg of CPE or 50 µg of CPE plus 1 mM of mepacrine. The intestinal loops were collected after one hour of incubation and assessed by microscopic evaluation. Mepacrine significantly reduced the severity of microscopic lesions. Ligated intestinal loops in another group of mice were inoculated with 100 µg of CPE only (a dose known to induce enterotoxemia) or 100 µg of CPE plus 1 mM of mepacrine. After four hours of incubation, lethality was recorded. The results of this experiment indicated a significant protection against CPE-induced enterotoxemia in those mice receiving mepacrine. Finally, multiple ligated small intestinal loops were prepared in rabbits to test the effect of different CPE and mepacrine concentrations after 6 hours of incubation. Fluid accumulation in the loops was recorded and samples were collected for histology. Although no differences in fluid accumulation were seen in the loops of rabbits treated with or without mepacrine, histological protection was seen in the loops co-inoculated with 50 µg of CPE and 1 mM of mepacrine. Taken together, these results indicate a protective role of mepacrine against CPE-induced enterotoxemia in mice and intestinal pathology in both mice and rabbits, suggesting that this drug is a potential therapeutic candidate for treating CPE-mediated gastrointestinal diseases.

*PII-3*

## COMPARATIVE PATHOGENOMICS AND PHENOTYPE ANALYSIS OF *CLOSTRIDIUM TERTIUM* AND *C. PARAPUTRIFICUM* ISOLATED FROM COLOMBIAN CLINICAL SAMPLES DURING *C. DIFFICILE* SCREENING

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Three Colombian fecal isolates were identified as *Clostridium paraputrificum* (Cpar; Gcol.A11) and *C. tertium* (Cter; Gcol.A2 and Gcol.A43), respectively, during a screening scheme for *C. difficile* (Cdif). An inter- and intra-taxa comparative analysis was carried out, considering database genomes to determine their taxonomic relationships and main virulence factors. The highly diverse Cpar genomes contained a large number of accessory genes. The toxZ, which was present in all species genomes is reported here for the first time and was the main virulence factor. Cdif toxA and toxB toxin homologs and other possible virulence factors were also identified. The genetic diversity and accessory gene percentage observed among Cter genomes was lower than that for Cpar. The only virulence factor among all Cter genomes was the EndoA interferase, a toxic component of the type II toxin-antitoxin system. The toxA was only detected in Gcol.A43, the Colombian isolate with higher experimentally verified cytotoxic effect. Higher sporulation efficiencies were experimentally determined for Gcol. A2 and Gcol.A43 than for Gcol.A11 (84.5%, 83.8% and 57.0%, respectively), a finding supported by the greater number of proteins associated with sporulation pathways found in the Cter genomes compared with Cpar (33.3 and 28.4 in average, respectively).

# *CLOSTRIDIUM BOTULINUM*: A SEVEN YEAR METHOD COMPARISON OF DIAGNOSITIC SPECIMENS

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Clinical, agricultural and public health laboratories screen thousands of samples daily for bacterial agents and toxins. Sample preparation, processing and analysis using conventional microbiological methods can range from days to weeks for pathogen identification. An agent of high concern is Botulinum neurotoxin (BoNT) which cause the disease known as botulism by inhibiting neurotransmitter release at the neuromuscular junction. In recent years, our lab has seen an increase in botulism related illnesses. As the number of specimens increases, there exists a need to quickly identify exposure sources which has led our lab to explore numerous assays for BoNT detection including the use of polymerase chain reaction (PCR), mouse bioassay (MBA), whole genome sequencing (WGS) and Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF/MS). A CDC developed endopeptidase-based MS method, was transitioned to be used with the Bruker Daltonics Biotyper in our lab. This method uses the toxin activity to identify all BoNT types (A-G) by specifically cleaving peptides. Toxins are identified by the mass-to-charge ratios of these fragmented peptides. Specimens suspected of containing BoNT are tested with an in-house developed rtPCR and MS assays. Subsequently, through WGS, isolates of BoNT-producing organisms are sequenced using Nextera XT library preparation kits and 500 cycle MiSeq reagent cartridges. SNP-based analysis was used to examine closely related isolates and provide information on toxin production and type. With an assay sensitivity for BoNT types ranging from 0.3-25 $mLD_{50}$ for MS and 20 gc for the toxin producing organism by rtPCR, comparative data for each assay will be highlighted from the past seven years to assess detection capability; including direct comparative studies of the MS assay with the gold standard method (MBA). Our findings support identification of BoNT using the MS method, replacing the need for performing traditional costly, time-consuming methods while eliminating animal testing. Additionally, with these methods a definitive link between positive patient specimens to their environmental or food source is possible.

*PII-5*

## ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF CLOSTRIDIUM SPECIES ISOLATED IN A UNIVERSITY HOSPITAL IN TURKEY

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*Clostridium* species cause life-threatening infections. Therefore, providing recent antimicrobial susceptibility data of these isolates is very important for effective empirical treatments.

**Objective:** We aimed to investigate antimicrobial susceptibility profiles of various *Clostridium* species isolated from invasive infections in Marmara University Hospital.

**Methods:** In total 31 *Clostridium* clinical isolates, collected between January 2011 to December 2017 in routine diagnostic procedures in a Turkish university hospital, were tested. The isolates were identified by using MALDI-TOF mass spectrometry (VITEK MS, bioMerieux, France). MICs of 11 antibiotics were determined using E-test methodology (bioMerieux, France) and the CLSI guidelines (M11-A7) were used for interpretation.

All *Clostridium* isolates belonging to 13 species were isolated from blood, sterile body fluid, biopsy, abscess and needle aspirate. Number of species were *C. perfiringens*, 7, *C. septicum*, 5, *C. ramosum*, 4, *C. tertium*, 3, *C. paraputrificum*, 3, *C. butyricum*, 2, *C. sordellii*, 1, *C. fallax*, 1, *C. glycolicum*, 1, *C. cadaveris*, 1, *C. sporogenes*, 1, *C. clostrdioforme*, 1, *C. difficile*, 1.

**Results:** All isolates were susceptible to metronidazole, amoxicillin-clavulanic acid, imipenem and meropenem. Susceptibility rates of cefoxitin, ampicillin, chloramphenicol, clindamycin, moxifloxacin were 83.8%, 77.4%, 83.8%, 54.8%, 74.1%, respectively. The $MIC_{50}$ and $MIC_{90}$ values were 1.5-2 mg/L for vancomycin and 2-0.125 mg/L for tigecycline .

**Conclusion:** According to our data, metronidazole, combinations of beta lactam-beta lactamase inhibitors and carbapenems seem to be effective on *Clostridium* species. However, resistance to clindamycin, moxifloxacin, cefoxitin and chloramphenicol ranged from 38% to 15%. This should be kept in mind when choosing antibiotics for the empirical treatment of infections caused by *Clostridium species.*

***1300*** ***POSTER SESSION II:*** **PROBIOTICS**



Posters will be presented in Poster Session II
Thursday, July 12 1300-1400.

*PII-6*

## STUDY OF SOME PROBIOTIC TRAITS OF CLINICAL ISOLATES OF ORAL STRAINS OF *STREPTOCOCCUS SALIVARIUS*

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The *Streptococcus salivarius* K12 strain is the first oral probiotic widely used in the treatment of halitosis and the maintenance of good oropharyngeal health. However, only very few studies have been carried out on the effect of autochthonous oral strains of streptococci on species implicated in oral diseases such as dental decays or periodontitis.

The purpose of this study was to investigate whether clinical isolates of oral strains of *Streptococcus salivarius* showed some probiotic capacities against oral pathogenic species.

For this, thirty-two clinical strains of streptococci were tested. The *S. salivarius* K12 strain was used as positive control. All determinations were performed in triplicate. Antibacterial activity was carried out on two cariogenic strains (*Streptococcus mutans, Scardovia wiggsiae*) and two periodontopathogenic strains (*Porphyromonas gingivalis, Fusobacterium nucleatum*) with the agar overlay technique. The adherence capacity was tested on glass surface. Adherence was scored from 0 (no adherence) to 4 (firmly adherent). The genes of salivaricins A and 9 were searched using a PCR method.

Twenty-six strains formed a firmly adherent biofilm. As compared with the control strain, twenty-two of our strains showed an overall antibacterial activity greater than that of this probiotic. All the strains had an antibacterial activity against *F. nucleatum* and *S. wiggsiae*. The latter was the most sensitive species. Two *S. salivarius* strains were positive for the gene of salivaricin 9, and two others for the gene of salivaricin A.

This study has shown that oral clinical species of *Streptococcus salivarius* seem to be an interesting way to fight against oral pathogens.

These results provide us with new research perspectives. It would be, for example, particularly interesting to study the mechanisms of inhibition of S. *salivarius* within a dynamic biofilm to better understand its possible mechanisms of action in the dental plaque.

# DISCOVERY OF NATURAL PRODUCTS THAT PROTECT FROM *CLOSTRIDIUM DIFFICILE* PROTEIN TOXINS

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*Clostridium difficile* is a leading cause of antibiotic-associated diarrhea. Disruption of the normal gut flora by antibiotics allows *C. difficile* to proliferate and secrete protein toxins that destroy the colonic epithelial cells, leading to diarrhea and pseudomembranous colitis. Recent studies have shown that neutralizing toxin function is an effective strategy to prevent disease pathogenesis. There remains a great unmet medical need for agents that neutralize toxin function without disrupting the natural microbiota. Small molecule antitoxins would be an ideal treatment option of *C. difficile* infections because they can be administered orally, making them capable of inhibiting primary infections, and are also specific to the protein toxins, allowing the gut flora to recover and eliminating the possibility of recurrence. Currently, there are no small molecule therapeutics capable of neutralizing the toxins of C. *difficile*. To this end, we screened 701 different strains of Streptomyces, a bacterium that produces a diverse collection of small molecule secondary metabolites, and uncovered extracts from five different strains that can protect mammalian cells from intoxication by toxin B. Remarkably, these active compounds can inhibit the *C. difficile* protein toxin yet remain nontoxic to mammalian cells even at 1000 times the effective inhibitory concentration. Preliminary results indicate that these molecules act on the host and prevent toxin uptake. The discovery of a safe, host specific, compound against the *C. difficile* protein toxins holds the potential of becoming an effective treatment option for disease.

*PII-8*

## *IN SILICO* RISK ASSESSMENT AND *IN VITRO* SUSCEPTIBILITIES TO ANTIBIOTICS OF THREE STRAINS OF BACTERIA CONTAINED IN A COMMERCIAL PROBIOTIC (BIO-K+) USED AS AN ADJUNCT DURING ANTIBIOTHERAPY

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A probiotic product containing three specific strains of lactobacilli is frequently used as an adjunct to antibiotic treatment in order to prevent *Clostridium difficile* infections (CDI), so the profile of its intrinsic and transferrable antimicrobial resistance is of interest to stewardship teams. The genome sequences for *L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 were analysed *in silico* to determine the risk of transferring resistance and to confirm the safety of these strains. None of the 2,353 sequences, from the Virulence Factor Database were detected, representing 408 virulence factors and 24 pathogenicity islands in either genome. Over 250 antimicrobial resistance genes targeting the major classes of antimicrobial agents were screened. A limited number of motifs related to antibiotic resistance were detected but the genes were not sufficiently complete to confer full resistance. Moreover, no plasmid was detected in the three strains, thus limiting the possibility to transfer unknown genes of resistance to antibiotics. The susceptibility to over 30 antibiotics was measured directly *in vitro* using Etest. Each strain was sensitive to multiple antibiotics, resistant to sulfamethoxazole and metronidazole and had elevated minimal inhibitory concentration (MIC) against kanamycin and streptomycin. *L. casei* LBC80R and *L. rhamnosus* CLR2 were also resistant to vancomycin. Multiple quinolones, including ciprofloxacin, showed an elevated MIC against *L. acidophilus* CL1285. *L. rhamnosus* CLR2 had exceptionally high resistance to erythromycin, possibly explained by a single mutation in the region V of the 23S rDNA. These analyses confirm that each of the three strains is sensitive to multiple antibiotics, resistant to the antibiotics commonly used to treat *C. difficile* infections, and does not pose a liability for transferring these forms of resistances to other bacteria. Overall, these molecular and laboratory results suggest that the probiotic Bio-K+ is safe to use as an adjunct to antibiotics and poses a minimal risk of transferring genes of resistance.

# EFFECT OF KEFIR STRAINS IN THE GROWTH OF ANAEROBIC BACTERIA

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Probiotics, by classical definition, are living organisms that when ingested in adequate amounts, promote benefits to the individual. Currently, there are several commercial probiotics, which are widely used both in the prevention and treatment of diarrhea associated with antibiotic therapy and in the treatment of infections by pathogens such as *Clostridium* sp.. These probiotic microorganisms help to colonize, even temporarily, to modulate the immune system and some are able to produce bacteriocins, preventing the infection. Kefir is a fermented probiotic product obtained from the fermentation of milk by kefir grains. The aim of this study was to evaluate the effect of *Lactococcus lactis* subsp. *lactis* and *Lactobacillus paracasei* strains isolated from kefir in anaerobic bacteria. For the antimicrobial activity assay, the isolates were tested using the spot-on-the-lawn method. Strains of *L. paracasei* and *L. lactis* subsp. *lactis* were cultivated in MRS broth for 24 h, while, *Bifidobacterium dentium*, *Bacteroides fragilis*, *Fusobacterium nucleatum*, *Clostridium citroniae*, *Clostridium scindens* and *Clostridium difficile* (ribotypes 630, 20291, 014, 015, 027, 133, 142, 143 and 106) were cultured in BHI broth for 24h. After adjustment of $OD._{600}$ to 1.0, the bacteria were seeded confluently on blood agar agar plates supplemented with hemin and menadione. Then, spots of 10 µl of the culture of *L. paracasei* and *L. lactis* subsp. *lactis* were innoculated. We observed that *L. lactis* subsp. *lactis* was able to inhibit the growth of *C. difficile* ribotypes (with exception of ribotype 630). The assay performed using *L. paracasei* did not show any inhibition halo in the tested strains. These results show the potential use of this strain in the prevention or treatment of *C. difficile* infection, but further studies are still needed.

Financial support: Capes, CNPq, FAPERJ

*PII-10*

## INTERFERENCE OF PREBIOTICS IN THE GROWTH AND BIOFILM PRODUCTION OF ANAEROBIC BACTERIA

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The microbiota is essential for health maintenance, preventing the establishment of pathogens such as *Clostridioides difficile*. *Clostridium citroniae* and *Clostridium scindens* are bacterial components of the gut microbiota that may act as a barrier to infections caused by pathogens. Fructooligosaccharides (FOS) and inulin are soluble fibers widely used as prebiotics associated with stimulating the growth of beneficial intestinal bacteria. The present study aimed to evaluate the interference of prebiotics inulin, FOS and the combination of these in the growth of *C. citroniae*, *C. scindens* and *C. difficile* ribotypes hypervirulent 027 and 135, besides the analysis of the biofilm production and interference of prebiotics of the same. Strains were inoculated in microplates containing the three different conditions quoted in varying concentrations of 1% to 8%, and the growth was monitored in an ELISA reader using the optical density of 620 nm for 24 hours. Results revealed the ability to reduce the growth of *C. difficile* strains by the prebiotics tested. Through assays to verify biofilm production on microplates, the *C. difficile* strains tested were categorized as a strong producer (027) and a moderate biofilm producer (135). *C. citroniae* and *C. scindens* were categorized as low biofilm producers by the method used. The *C. difficile* 027 strain showed no significant difference in biofilm production in the presence of *C. citroniae* and *C. scindens*. However, *C. difficile* strain of ribotype 135 showed a reduction in biofilm production when co-cultivated with *C. citroniae*.

Financial support: PIBIC-UFRJ, Capes, CNPq, FAPERJ

# SPECIATION OF LACTOBACILLI ISOLATES OF NORMAL FLORA BY BIOCHEMICAL CHARACTERIZATION & MALDI TOF ANALYSIS

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**Purpose:** Since Lactobacilli constitute an important part of the normal gastrointestinal endogenous flora and a regulator of the vaginal ecosystem in females of reproductive age, an attempt was made to identify the species of Lactobacilli isolated from vagina and evaluated morphological, cultural, biochemical characteristics and rapid MALDI-TOF analysis for species identification in this study.

**Methods**: A total of 120 high vaginal swabs were collected in MRS (deMan Rogosa Sharpe) broth and stool samples were collected in Robertson's cooked meat medium and subcultured after 24 hours of incubation, onto MRS agar and 5% sheep blood agar. Anaerobic incubation was carried out in Gas Pak Jar at $37^0$C for 48-72 hours. Identification of the isolates were done by Gram stain, colony morphology, biochemical characteristics according to the Wadsworth manual. 30 randomly selected strains were subjected to MALDI ToF analysis using Bruker Biotyper.

**Results**: Out of the 101 isolates, the predominant Lactobacillus species were assigned to *Lactobacillus acidophilus* (43), *L. iners* (18) *L. fermentum* (11), *L. vaginalis* (9) *L. jensenii* (8) *L. rhamnosus* (6), *L. gasseri* (4) and *L. paracasei* (2). MALDI TOF also revealed concordant results except in 2 cases.

**Conclusion**: *L. acidophilus* is the major component of vaginal normal flora. MALDI TOF analysis proves to be a rapid tool for identifying the Lactobacilli isolates up to species level, which is possible within one hour while conventional biochemical characterization requires a minimum 48 hours.

*PII-12*

## STRESS RESPONSE OF *LACTOBACILLUS PLANTARUM* D3 WITH POTENTIAL ANTI-OBESITY ACTIVITY

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Obesity is known as one of the major causes of illness in modern society. Probiotics lactobacilli with potential anti-obesity activity are of significant interest. We screened our lactobacilli collection for strains with potential anti-obesity activity. Among 49 lactobacilli isolated from shellfish (29) and vagina of Korean women (20), 5 strains showed over 50% anti-lipase activity. During live cell probiotics formulation, stress resistance is an important factor for cell viability. Thus, responses of the 5 strains to various stresses (the bile salts, acid stress, and freeze-drying stress) were examined. With 5 selected strains, survivability upon freeze-drying stress and acid / bile salt resistance were measured. Among the 5 strains, D3 showed highest stress resistance. D3 was molecularly identified as *Lactobacillus plantarum* by 16S rDNA sequencing. Upon freeze-drying stress test D3 survived up to 5*107 CFU/ml from the initial 6*108 CFU/ml. D3 was also found acid resistant (survived more than 50% at pH 2.5) and bile salt resistant (survived more than 3% at 0.3% bile salt). Interestingly, when long-term storage experiment was conducted after suspension of cells in distilled water at 25°C, D3 maintained the cell viability of 4*105 CFU/ml even after 1 month from the initial 6*108 CFU/ml whereas other strains were not viable. Intestinal adherence, an essential function of probiotics, has been known to have correlations with the auto-aggregation and hydrophobicity of the cell surface. Through cell surface hydrophobicity (CSH) and auto-aggregation, we measured potential adhesiveness of D3. D3 exhibited significantly higher percentage adhesion to all organic solvents than other strains. In CSH assay, D3 showed affinity to chloroform (81.7%), ethyl acetate (23.8%) and hexadecane (24.2%) with the highest affinity for chloroform, an acidic solvent. In auto-aggregation assay, D3 showed the highest 67% auto-aggregation after 5 h standing. Further studies are underway to substantiate the potential anti-obesity activity of D3 and to improve stress resistance for live cell probiotics formulation.

***1300*** ***POSTER SESSION II: CLOSTRIDIUM DIFFICILE:* PATHOGENESIS**



Posters will be presented in Poster Session II
Thursday, July 12 1300-1400.

Posters will be presented in Poster Session II
Thursday, July 12 1300-1400.

Posters will be presented in Poster Session II
Thursday, July 12 1300-1400.

*PII-13*

## CHARATERIZATION, MIXTURE IDENTIFICATION, AND STRAIN LEVEL RESOLUTION FOR *CLOSTRIDIOIDES DIFFICILE* DIRECT FROM CLINICAL SPECIMENS

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*Clostridioides difficile* (*C. diff*) is the most common hospital associated diarrheal pathogen in the United States with a U.S. economic burden of 5.4 billion dollars annually. Clinical diagnostics typically focus on a presence/absence toxin detection, but miss clinically-relevant information such as antimicrobial resistance profiles. Whole genome sequencing (WGS) has been used to characterize *C. diff* strains, but *C. diff* is difficult to grow in the laboratory due to its anaerobic lifestyle. An alternative to WGS is amplicon sequencing, where a multiplex polymerase chain reaction (PCR) amplifies targets directly from a clinical specimen, removing the need to culture and providing reproducible data in a rapid and inexpensive assay. To test the viability of this approach, DNA was extracted from fecal clinical samples and two multiplex PCR assays were amplified from the extractions, pooled, and sequenced. One of the multiplexes targets a *C. diff* species identification target, an informative phylogenetic target, and other targets associated with virulence and antimicrobial resistance. The other multiplex targets nine *C. diff* single nucleotide polymorphism (SNP) loci that can be used for strain level resolution and source attribution. The sequencing data was analyzed with the Amplicon Sequencing Analysis Pipeline (ASAP), whose results provide a profile of virulence and resistance genes and also produces SNP profiles that can be used for mixture deconvolution. Using the *WG-FAST* pipeline, targeted SNPs from amplicons are used to place the strains in a global phylogeny, providing strain level resolution that can be used for source attribution or contact tracing. This approach represents an inexpensive, rapid assay that provides clinically-actionable data directly from clinical specimens containing *C. diff*.

# RIBOTYPE DIVERSITY OF TOXIGENIC *CLOSTRIDIUM DIFFICILE* WITH HOUSEFLIES AND ANIMAL FECES

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**Background:** Spores of *C. difficile* can survive and disseminate in any environs and act as sources for human and animal colonization/infections. Although likely ubiquitous worldwide, the ecology and prevalence of *C. difficile* spores in the animal farm environment is poorly understood. The objectives of the study are to isolate and characterize *C. difficile* from houseflies and animal feces in Sabah, Malaysia.

**Method:** As part of a world-wide surveillance effort, we collected houseflies from horse (n=110) and cattle farms (n=137), horse fecal samples (n=97) and deer fecal samples (n=103). Samples were assessed for *C. difficile* using anaerobic enrichment culture and molecular methods. Suspected colonies from cycloserine cefoxitin fructose agar (CCFA) plates were characterized by multiplex PCR (*tcdA, tcdB, cdtA, cdtB, and tpi* genes) and strain typed using fluorescent PCR ribotyping technique.

**Result:** A total 17 of 247 (6.9%) housefly samples were culture positive for *C. difficile* of which 5.7% (15/247) samples were toxigenic *C. difficile.* A total of 8 distinct ribotypes (F002, F107, F014-020, F053-163, F097, FP470, FP483, and F027) were identified from 15 *C. difficile* isolates tested. Among the horse fecal samples 10.3% (10/97) were positive for *C. difficile* (9.3% samples for toxigenic strains). Four unique ribotypes (F002, F017, F027, and F78-126) were detected from the nine fecal isolates. Among the deer samples, 41.7% were *C. difficile* (12.6% toxigenic) positive.

**Conclusion:** We identified a high prevalence of toxigenic *C. difficile* with diverse ribotypes from animal farm fecal and housefly samples in Sabah, Malaysia. Our findings suggest that animal feces might be the source of *C. difficile*, and houseflies can be considered as a potential vector of transmission among animals.

*PII-15*

## DETERMINING THE ROLE OF THE SpoVAD AND SpoVAE GENES IN DPA RELEASE DURING *CLOSTRIDIUM DIFFICILE* SPORE GERMINATION

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The *Clostridium difficile* vegetative form is strictly anaerobic and susceptible to antibiotic treatment. However, the spore form is extremely resilient and can survive outside the body under aerobic conditions, is resistant to most disinfectants, and has a significant resistance to extreme temperatures. Spores have considerable heat resistance due to the large amounts of pyridine-2,6-dicarboxylic acid (dipicolinic acid [DPA]) in the spore core. During spore formation, DPA is packaged into the developing spore resulting in the displacement of water. Upon the initiation of spore germination, this DPA is released and the core is rehydrated. In *Bacillus subtilis*, a model organism for studying sporulation and germination, the proteins encoded by *spoVA* operon (SpoVAA-AB-AC-AD-AEa-AEb-AF) play a role in DPA release, but *C. difficile* encodes only 3 orthologues: *spoVAC*, *spoVAD*, and *spoVAE*. Previously, we determined that SpoVAC plays a role in DPA release from the spore core in mechanosensing fashion, but the roles of *spoVAD* and *spoVAE* are unclear at this time. In order to study the roles of these two genes, we will create mutants using the *pyrE* allele-coupled exchange and / or our recently-developed CRISPR-Cas9 system. Preliminary data suggests that *spoVAD* is important for DPA packaging into the spore core, similar to what is observed in *B. subtilis*. Understanding the roles of SpoVAD and SpoVAE in DPA packaging and / or release may lead to better understanding of one of the first steps in spore germination of this medically important microorganism.

# PHOSPHORYLATION AND FUNCTIONALITY OF CDTR IN *CLOSTRIDIUM DIFFICILE*

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The aim of this study was to identify the phosphorylation site of the virulence regulator CdtR in *Clostridium difficile* R20291, and establish the functionality of its PCR-ribotype 078 homolog.

We generated dephosphomimetic and potentially-phosphomimetic CdtR-encoding variants in which our predicted phosphoAsp residue had been substituted with Ala and Glu respectively. We also amplified *cdtR* from the archetypal ribotype 078 strain M120, containing 9 non-synonymous mutations including a premature truncation, and integrated all three *cdtR*-variants at the *pyrE* locus in R20291ΔPaLocΔ*cdtR*.

The Asp-Glu CdtR-expressing strain mirrored the *cdtR*-null parental strain, in which CdtA was reduced to levels undetectable by Western blot. Conversely, the Asp-Glu CdtR phosphomimic was at least partially functional, as CdtA was detectable for this mutant, although the level of production was considerably lower than its wild-type counterpart. M120-derrived CdtR was again comparable to the *cdtR*-null parental indicating its lack of functionality. To ensure that this observation stemmed from the mutations within the coding region and not the polymorphic promoter region, we tested the capability for *E. coli* to tolerate chloramphenicol using R20291 and M120-derrived *cdtR* promoters to drive CatP expression. Our data indicated that both constructs drove constitutive expression. Finally, we amplified *cdtR* from seven ribotype 027 and five ribotype 078 clinical isolates from our culture collection and sequenced their open reading frames. Nucleotide sequences were 100% identical to their archetypal strains within each ribotype with a 4.4% divergence between ribotypes.

Our preliminary data provide valuable insights into the activation of functional CdtR and the non-functionality of the ribotype 078 homolog.

*PII-17*

## EFFECT OF HUMAN GUT METABOLOME ON *CLOSTRIDIOIDES DIFFICILE* VIRULENCE GENE EXPRESSION

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*Clostridioides difficile* is an important nosocomial pathogen, being considered one of the major etiological agents of antibiotic-associated diarrhea (AAD). Treatment of *C. difficile* infection with antibiotics further increases the impact on the gut microbiota. Therefore, alternative treatments to eradicate *C. difficile* while preserving the gut microbiota are necessary. In this context, secondary metabolites from the intestinal microbiota emerge as a promising source of bioactive compounds. The aim of this study was to analyze the effect of small molecules extracted from human feces on the expression of virulence genes from *C. difficile*. Fecal samples were collected from healthy donors without prior use of antibiotics for the past six months. Small molecules were extracted from fecal samples using ethyl acetate. Dried extracts were resuspended directly into TPG culture medium, filtered, and the pH was adjusted to match that of culture medium alone. Dried residues of pure ethyl acetate were used as controls. The fecal extract slightly inhibited the growth of *C. difficile* R20291. After approximately 6 hours of growth, the cultures had reached the mid-logarithmic growth phase and RNA was isolated. The yield of RNA obtained from cultures exposed to fecal extracts was higher than the RNA obtained from controls, suggesting that the fecal extract could be directly affecting the cell wall composition. Alternatively, the fecal extract could be affecting the expression of genes associated with cell wall synthesis. The effect of the fecal extract on the global gene expression profile of *C. difficile* R20291 shows that 97 genes including phage-related genes, genes involved in the degradation of amino acids and purine ribonucleotide biosynthesis were activated while 198 genes such as genes associated to chemotaxis and motility, transport of carbohydrates, organic acids and alcohols and cell membrane composition are repressed ($P < 0,05$). Other tests will be performed to further investigate the role of gut small molecules on virulence gene expression.

## DIET DRIVEN SELECTION OF EPIDEMIC, HYPERVIRULENT RIBOTYPES OF *CLOSTRIDIUM DIFFICILE*

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*Clostridium difficile* disease has markedly increased since the early 2000s, becoming a dominant nosocomial pathogen in North America and Europe. While this increase can be attributed to a small number of pathogenic ribotypes, little is known about the mechanism underlying their epidemic and hypervirulent nature.

Here we show that two phylogenetically distinct *C. difficile* ribotypes (RT027 and RT078) have independently acquired unique mechanisms to metabolize low concentrations of the disaccharide trehalose. Ribotype 078 strains have independently acquired a cluster of four genes encoding proteins annotated to participate in trehalose metabolism and transport. One of these genes, a PTS permease, is both necessary and sufficient for growth on low concentrations of trehalose. RT027 strains contain a single nucleotide polymorphism resulting in a L172I mutation in the trehalose repressor (TreR), increasing their sensitivity to trehalose by >500 fold. Furthermore, dietary trehalose increases the virulence of a RT027 strain in a mouse model of infection, indicating that trehalose metabolism plays a role in disease severity. To empirically show that the *treR* mutation is responsible for enhanced trehalose metabolism and disease severity we are creating an isogenic I172L reversion using CRISPR technology. This strain will be tested alongside the wildtype strain for trehalose metabolism, virulence, and whole transcriptome shotgun sequencing. These results will help elucidate the role of TreR and trehalose metabolism in the pathogenicity of *C. difficile*.

*PII-19*

## FACTORS AFFECTING BILE SALT GERMINANT SENSITIVITY DURING *CLOSTRIDIUM DIFFICILE* SPORE GERMINATION

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*Clostridium difficile* is a Gram-positive spore-forming anaerobic bacterium that is the leading cause of antibiotic-associated colitis and gastroenteritis-associated death globally. There are ~500,000 cases in the U.S. yearly with an associated treatment cost of >$3 billion. To initiate infection, the extremely resistant aerotolerant spores must germinate in the gut of a susceptible host. While nearly all spore-forming bacteria use transmembrane germinant receptors to trigger germination, *C. difficile* uses the CspC pseudoprotease to sense bile salt germinants. When CspC senses germinant, it activates the subtilisin-like protease CspB, which proteolytically activates SleC, the cortex hydrolase. When activated, SleC degrades the protective spore cortex layer, which is an essential step in the germination pathway. We previously showed that CspC function and incorporation into spores is dependent on a related pseudoprotease domain, CspA. While Csp family proteins play a crucial role in spore germination, it remains unknown how Csps are incorporated into spores. In this study, we determined that CD0311 (renamed GerG), a previously uncharacterized hypothetical protein, modulates the incorporation of the Csp germination regulators into spores. The reduction in Csp levels in *gerG* spores was associated with increased germination heterogeneity in single-spore germination assays and reduced responsiveness to bile sale germinants. We have further identified mutations that specifically decrease CspC levels in spores, which also correlates with reduced germinant sensitivity. Taken together, our analyses support a model in which CspC levels determine the responsiveness of *C. difficile* spores to bile salt germinants. The mutations described here will facilitate future studies directed at testing the impact of reduced germinant sensitivity on *C. difficile* infection *in vivo*.

# LOCALIZATION OF *CLOSTRIDIUM DIFFICILE* VEGETATIVE CELLS AND SPORES DURING AND AFTER INFECTION

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*Clostridium difficile* infection initiates with the ingestion of dormant spores which subsequently germinate, colonize the lower GI tract and cause disease. The interactions between the host mucosa, and *C. difficile* cells and spores during and after active infection remains incompletely understood. To analyze this interaction in infected mice, we visualized *C. difficile* vegetative cells and spores in the colon and cecum during active infection (2-10 days post-infection) and after active infection (15-40 days post-infection). To identify vegetative cells, we analyzed fixed and sectioned GI tract tissue by fluorescent *in situ* hybridization (FISH), using a *C. difficile* specific 16S rRNA probe. We detected spores by immunofluorescence microscopy (IFM) with polyclonal anti-SlpA antibodies. These experiments demonstrated that, as we previously showed for vegetative *C. difficile* cells during active infection, vegetative cells and spore-like cells are located within mucin-associated communities both during and after active infection. These communities were present in the outer mucus layers of the cecum and colon. To quantify the number of spores in the lumen and the number associated with the mucus layer, we harvested luminal and mucosal fractions from infected animals at various times during and after infection, through day 40 post-infection and quantified the numbers of colony forming units before and after heat-killing. We found spores and vegetative cells were present at all time points, and that more spores were present in the cecum than the colon during and after active infection. These data provide new information on the locations of *C. difficile* cells, and in particular spores, in the GI tract during and after active infection. This information could provide insight into a role for the spore in recurrence of disease.

*PII-21*

## IDENTIFICATION OF GENETICALLY DISTINCT *CLOSTRIDIUM DIFFICILE* ISOLATES IN HOUSTON

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*Clostridium difficile*, a CDC threat level urgent pathogen is associated with significant antimicrobial resistance, which has lead to worldwide outbreaks. Specifically, the recent pandemic caused by ribotype 027 was the result of dissemination of two separate lineages resistant to fluoroquinolones called the fluoroquinolone resistant 1 (FQR1) and FQR2 lineages. Both lineages originated in North America, however, specific mapping within the US and Canada has yet to be done. We sought to determine whether these pandemic strains and specific lineages were also present in Houston, Texas. Given the international nature of Houston and denseness of hospital centers, we hypothesized that both lineages were present. To test the hypothesis, we sequenced 61 ribotype 027 clinical isolates. DNA was purified and sequenced on an Illumina NextSeq. R20291 (ribotype 027) was used as the reference genome for alignment and mapping and established FQR1/FQR2 strains were used to help identify the lineages. Whole-genome SNP analysis was performed using 292 SNPs, of which 222 were non-synonymous mutations, 36 were synonymous substitutions, and 34 were intergenic changes. Phylogenetic analysis separated the strains into two prominent groups, which grossly differed by 21 SNPs. 30 isolates clustered with other FQR2 lineage worldwide strains, 30 isolates were identified as the FQR1 lineage, and one strain was found to be pre-epidemic. Of note, phylogeny demonstrated unique clustering amongst the Houston strains indicating geographic source has defined the local 027 genetics. Other antimicrobial resistance genes were also present amongst the strains including *ermB* mutations (n=34), *tetM* mutations (n=4), *aac6* mutations (n=3), and *tem* mutations (n=1). Collectively, this data demonstrates that both FQR lineages are present in Houston and have become uniquely defined.

# ANTIBIOTIC TREATMENT INDUCES THE FORMATION OF NON-SPORE *CLOSTRIDIUM DIFFICILE* PERSISTER-LIKE CELLS

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One of the main complications of *Clostridium difficile* infections (CDI) is its high recurrence rate, reaching 20, 40 and 50% in a first, second and third episode. *C. difficile* spores, resistant to antibiotics and host immunity plays an essential role in CDI. The alteration of the microbiota contributes to the germination of spores of *C. difficile* and the colonization through mechanisms not completely elucidated. Additionally, there are mechanisms that would contribute to a rapid growth of *C. difficile* after antibiotic treatment, and that should be involved in the dynamics of *C. difficile* populations to overcome the effect of antibiotics and cause CDI or recurrent CDI. In this context, it is possible that a phenotypic switch associated with the emergence of persistent *C. difficile*, tolerant to antibiotics, would provide an advantage in growth compared to the microbiota after antibiotic treatment. The aim of this work is to demonstrate that lethal antibiotic concentrations induce the appearance of persister-like non-spore cells in *C. difficile*. Wild-type and derivative *spo0A* mutant strains were tested for their susceptibility antibiotics, as determined by an agar microdilution test. The *C. difficile* 630 and R20291 were only sensitive for vancomycin. Persister cells generation were determined for all strains using up to 10X MIC for every antibiotic for up to 6 days. Briefly, it was grown anaerobically in BHIS medium at 37°C O.N and treated with each antibiotic, aliquots were taken at several hours up to 6 days and plated on agar-BHIS medium. This was repeated at least 3 times. The persistent ones were confirmed by the MIC determination at the end of the experiment, which was equal to that of the parental strains used at the beginning. We were able to induce the formation of persister-like behaviors since biphasic killing curves could be observed in response to treatment antibiotics. This work provides, for the first-time, experimental evidence of the appearance of persister-like cells in *C. difficile* opening a new research avenue in the pathogenesis of this nosocomial pathogen.

*PII-23*

## EMERGING HYPERVIRULENT EPIDEMIC *CLOSTRIDIUM DIFFICILE* STRAIN OF ST37 TYPE (TOXIN A-B+) POSES A POTENTIAL THREAT IN CHINA

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**Object:** *Clostridium difficile* strains of ST37 type (RT017) are most frequently isolated epidemic isolates in China. We aimed to compare major virulence factors of an epidemic *C. difficile* isolate of ST37 type from China with those of *C. difficile* RT20291 and CD630.

**Methods:** The first hypervirulent XY-06 (ST37, A-B+) *C. difficile* strain was isolated from ICU hospitalized patient in China. The toxin production was measured by ELISA assay and commercial Kits. Cytotoxic effects of the strains on cultured cells were assessed by cell rounding assays. The pathogenicity in vivo was evaluated in mouse model of *C. difficile* infection (CDI). The resistance of the strain to antimicrobial agents was performed by micro-dilution assay. The spore adherence effects on human gut epithelial cells was performed by adherence assay. Genome of XY-06 strain was sequenced, and the entire toxin gene PaLoc was compared with 027 and CD630 strains.

**Results:** *C. difficile* XY-06 produced much more TcdB, adhered stronger to gut epithelial cells, in comparison with *C. difficile* RT20291 and CD630. In a mouse model of CDI, strain XY-06 was more virulent than strain CD603, and was comparable to strain RT20291. No sizable resistance to antibiotics tested was detected. In addition, biofilm formation, sporulation, germination and motility of this strain was also evaluated, in comparison with RT20291 and CD530, and no remarkable differences were noticed. These findings highlight the potential threat of epidemic strains ST37 in China.

# A HYPERVIRULENT NON-027, NON-078, BINARY-TOXIN POSITIVE *CLOSTRIDIUM DIFFICILE* ISOLATE FROM CHINA

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**Background:** *Clostridium difficile* strains expressing the binary toxin (CDT), is generally found in *C. difficile* 027 (ST1) and/or 078 (ST11) in clinic associated with severe *C. difficile* infection (CDI). However, we recently reported a CDI case infected by a binary toxin-positive, non-027, non-078 *C. difficile* LC693 which is associated with severe diarrhea in China. We aimed to compare the virulence factors and pathogenicity of *C. difficile* LC693 with those of *C. difficile* RT20291 and CD630.

**Methods:** The toxin production was measured by conventional ELISA assay and commercial Kits. The pathogenicity *in vivo* was evaluated in the mouse model of CDI. The sporulation capacity was measured by sporulation rate assay. The early step of germination was performed by monitoring the initiation of spore germination and Ca-DPA release. The motility was performed by swimming plate assay, and electron microscopy was performed to examine the presence/absence of flagellar structures on the surface of *C. difficile*. Biofilm formation was evaluated by measuring the biofilm biomass of *C. difficile* strains in 96-wells plate. The spore adhesion to human gut epithelial cells was also performed.

**Results:** TcdA production of LC693 was lower than both R20291 and CD630. But the TcdB production of LC693 was higher than CD630 and lower than R20291. LC693 had the highest sporulation rate compared with R20291 and CD630, and showed a fast germination rate (64%) within 8 min at the early step of germination. However R20291 was showed a more fast completed DPA release in 30min. LC693 exhibited a notable motility in swimming plate, and carries abundant flagellar on its surface which was demonstrated by Electron microscopy. LC693 developed a robust biofilm formation capacity and a high adherence ability on human gut epithelial cells. Finally, in a mouse mode of CDI, LC693 displayed a high pathogenicity.

**Conclusion:** These findings highlight the pathogenicity of binary toxin positive non-027 and non-078 *C. difficile* strains. The virulence was lower than 027 strains, however it has a high spore formation rate which attributes to a high transmission capacity in hospital environment. More strict prevention and control measures should be performed in hospital to curb transmission between different patients.

*PII-25*

## PERINATAL METHYL-DONOR DIET AND SUSCEPTIBILITY TO *CLOSTRIDIUM DIFFICILE* INFECTION IN F1 MICE

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Murine models investigating CDI have identified that antibiotics are required for colonization of *C. difficile* and subsequent disease. Due to an outbreak within our mouse facility, it was observed that CDI susceptibility was increased in F1 mice whose parents received a methyl-donor diet (MDD), compared to controls (mortality, 24.4% controls versus 76% MDD, $P = .03$), without the use of antibiotics. This observation led us to investigate if dietary alterations in the parental generation can lead to altered CDI susceptibility in F1 mice. We hypothesize that F1 mice whose parents received a MDD will display increased susceptibility to CDI resulted from an altered gut microbiota.

C57BL/6 breeders were fed a control or methyl rich diet, and all F1 mice were weaned on normal chow. F1 mice were challenged orally with spores from the outbreak strain (16N203), without prior antibiotic treatment. Nine days after, mice were treated with an intraperitoneal injection of clindamycin and re-challenged with 16N203. Mice were euthanized 48 hours following the second challenge. Disease was evaluated based on a murine clinical scoring system. Colon and cecum tissue samples were evaluated for histopathological features of disease. Community composition was determined through 16S rRNA gene sequencing.

Antibiotic administration was required for *C. difficile* colonization in F1 mice challenged with 16N203, independent of parental dietary intervention. Total clinical score was greater in MDD offspring (median = 9), compared to control (median = 4). Subscores for activity and posture, were higher in MDD offspring ($P = .001$ and $P = .002$, respectively). Preliminary histopathology analysis showed that MDD progeny displayed greater edema, increased neutrophil infiltration, and epithelial damage. Preliminary community analysis shows distinct differences between MDD and control diet progeny.

MDD supplementation in parental mice does not alter baseline susceptibility to *C. difficile* infection although, MDD offspring develop more severe disease when challenged after antibiotic administration. This increase in disease severity is associated with changes in the microbiota due to MDD supplementation.

# CHARACTERIZING THE DIFFERENTIAL GENE EXPRESSION PATTERN DURING GERMINATION AND OUTGROWTH OF *CLOSTRIDIOIDES DIFFICILE* SPORES

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The symptoms of *Clostridioides difficile* infection are caused by the action of two toxins secreted from *C. difficile* vegetative cells, TcdA and TcdB. Due to the strictly anaerobic nature of the vegetative form, the spore is considered to be the infectious form of *C. difficile* as a result of its ability to survive for extended periods of time in an aerobic environment. Because toxin secretion is necessary for infection, germination of the spore is considered one of the first steps in *C. difficile* pathogenesis. A greater understanding of the *C. difficile* spore germination process is important for generation of possible non-antibiotic / *C. difficile*-targeted therapies. In order to identify additional gene products whose functions are important during *C. difficile* germination and outgrowth we are performing RNAseq in order to analyze the whole transcriptome of dormant and germinating spores as well as ribosomal profiling of dormant spores. Following analysis of experimental data, mutants of highly expressed genes and genes whose messages are bound by ribosomes in dormant spores, will be generated. Following generation of mutants, phenotypes of each mutant will be analyzed to determine their role in *C. difficile* germination/outgrowth. If successful, identification of highly expressed gene products in dormant and germinating *C. difficile* spores will allow for the characterization of the factors responsible for initiating colonization within susceptible hosts. Moreover, genes identified to be important for *C. difficile* pathogenesis pathways may shed light into different approaches for treatment in the future.

*PII-27*

## DETERMINING THE INTERACTIONS OF GERMINANT PROTEINS CspA, CspC, AND SleC DURING *CLOSTRIDIUM DIFFICILE* GERMINATION

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*Clostridium difficile* infections are an increasing concern in the healthcare field where the widespread use of antibiotics has increased the prevalence of such infections. The difficulty in treating these infections lies with the ability of the microbes to produce spores. Spores are forms of bacteria that are resistant to many harsh environments and aid in the spread of *C. difficile* between hosts. Once in a host, the spore form must germinate to the actively-growing, toxin-producing vegetative form in response to a combination of certain bile acids and amino acids. In *B. subtilis*, a model organism for studying spore formation and spore germination, germination is triggered upon the recognition of germinants by germinant receptors embedded in the inner membranes of dormant spores. Though most of all studied endospore-forming bacteria encode orthologues of the ger-type germinant receptors found in *B. subtilis*, *C. difficile* does not. In prior work, we identified the germination-specific, subtilisin-like pseudoprotease, CspC, as the bile acid germinant receptor. *cspC* is encoded in an operon with *cspBA*. Upon translation, CspBA is processed into CspB and CspA. While CspC and CspA are pseudoproteases, CspB is a protease that processes the cortex hydrolytic enzyme, proSleC, to its active form during the imitation of spore germination. In our working model, CspC binds to its bile acid substrate and transmits the signal to CspB which then cleaves proSleC to its active form. We hypothesize that CspA, CspB and CspC exist as a complex within the *C. difficile* spore cortex. To test this hypothesis, we introduced a 6x histidine tag, using pyrE-mediated allelic exchange, into the 3' end of *cspBA*, *cspC*, and *sleC*. Experiments are ongoing to test the interaction of these proteins within the *C. difficile* spore. Determining the interactions of these proteins will provide a better understanding of the process of germination and could eventually be used as a target for anti-spore germination therapeutics.

# IDENTIFICATION OF *CLOSTRIDIUM DIFFICILE* IMMUNO-REACTIVE SPORE PROTEINS OF THE EPIDEMIC STRAIN R20291

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*Clostridium difficile* infections are the leading cause of diarrhea associated to the use of antibiotics. During infection, *C. difficile* initiates a sporulation cycle leading to the persistence of *C. difficile* spores in the host and disease dissemination. The development of vaccine and passive immunization therapies against *C. difficile* have focused on toxins A and B. In the present study, we used an immunoproteome-based approach to identify immunogenic proteins located on the outer layers of *C. difficile* spores as potential candidates for the development of immunotherapy and/or diagnostic methods against this devastating infection.

**Purpose:** Identify potential immunization target in the spore of *C. difficile* R20291.

**Experimental design:** To identify potential immunogenic proteins on the surface of *C. difficile* R20291, spore coat/exosporium extracts were separated by two-dimensional electrophoresis (2-DE) and analyzed for reactivity against *C. difficile* spore-specific goat sera. Finally, the selected spots were in-gel digested with chymotrypsin, peptides generated were separated by nanoUPLC followed by MS/MS using Quad-TOF-MS, corroborated by Ultimate 3000RS-nano-UHPLC coupled to Q-Exactive-Plus-Orbitrap MS.

**Results:** The analysis identified 5 immnoreactive proteins: spore-coat proteins CotE, CotA and CotCB, exosporium protein CdeC, and a cytosolic methyltransferase.

**Conclusion:** Our data provides a list of spore surface protein candidates as antigens for vaccine development against *C. difficile* infections.

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## *CLOSTRIDIUM DIFFICILE* STRAINS FROM THE MLST CLADE 2 INDUCE A DISTINCT PRO-INFLAMMATORY RESPONSE AND VIRULENCE POTENTIAL IN ANIMAL MODELS

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Previous phylogenomic analysis identified eight genetically distinct *C. difficile* clades, which 5 have been associated with humans and another 3 with the environment. The Clade 2 includes strains from the NAP1/R027/ST01 genotype, which have been associated to more severe CDI cases and outbreaks worldwide. To determine whether other strains from this clade are also highly virulent, we compared the pro-inflammatory response and the lethal activity induced by Clade 2 representatives from the sequence types ST01 (NAP1/RT027), ST67 (NAP1/RT019), ST41 (RT821), and ST252 using the murine ligated loop model and hamsters fed with spore suspensions, respectively. The tested strains showed notable differences in myeloperoxidase activity measurements, TNF-α, IL-1β, and IL-6 quantifications, histopathological analyses, and mortality rates, with the ST01 and ST067 isolates showing the highest virulence potential. This result seems to be related to toxin overproduction (ST01) or secretion of a variant TcdB (ST067).

# CHARACTERIZATION OF *CLOSTRIDIOIDES DIFFICILE* RYBOTYPES ISOLATED FROM STOOL OF DOMESTIC DOGS IN RIO DE JANEIRO, BRAZIL

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*Clostridioides difficile* is a common cause of enteritis in a variety of animal species and some reports have recently raised the importance of domestic animals as a reservoir of this pathogen for humans. So, the aim of this study was to give the first characterization to *C. difficile* strains obtained from pet dogs in Rio de Janeiro, Brazil. Thus, 151 samples of dog stools from 2014-2016, with or without diarrhea, were randomly selected regardless of gender, race or age (from 2 months to 18 years). Seven (4.6%) *C. dificile* strains were isolated (MzCd, RkCd, KsCd, PaCd, MnCd, RoCD and VrCd) and characterized phenotypically and genetically. PCR-Ribotyping revealed that most of the strains belonged to the following ribotypes: 106 (MnCd, MzCd and KsCd), and A+B+ for the *tcd*A e *tcd*B gene; two ribotype 010 (RkCd and RoCd) A-B+; and one ribotype 002 (PaCd) A-B-. The VrCd was untypable. None of the strains presented the binary toxin genes. The antibiotic resistance profile showed that 28.6% strains (KsCd and RoCd) were resistant to clindamycin (≥ 256µg/mL) and 28.6% strains (PaCd and VrCd) displayed full resistance to metronidazole (≥ 32 µg/mL). All strains were sensible to vancomycin, strong biofilm producers and showed great motility. Regarding the *tcd*C sequence of the toxigenic strains, two of them (MnCd and KsCd) presented deletions in a different region, 340 bp and 640 bp, from the NAP1/027, used for comparison. This first study conducted in Rio de Janeiro, Brazil about the isolation of *C. difficile* in domestic dogs showing the zoonotic potential of this pathogen. Isolation of *C. difficile* strains from dogs helps to study the pathogenesis of the disease, especially because two of isolates showed full resistance to metroinidazol, one of the recommended therapy choices for CDI, and allows a better understanding of epidemiological data in Brazil.

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## IDENTIFICATION OF SURFACE PROTEINS RESPONSIBLE FOR LAMININ-1 RECOGNITION IN *CLOSTRIDIOIDES DIFFICILE*

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*Clostridioides difficile* is recognized as the main cause of nosocomial diarrhea associated with antibiotics. The two main virulence factors of *C. difficile* are the production of the large toxins, TcdA and TcdB, which cause a severe inflammation in the colonic epithelium cells. *C. difficile* has also a repertoire of surface proteins involved in the adhesion to intestinal cells, within the colon colonization, but the ones responsible for the recognition of the extracellular matrix components (ECM) are still unclear. Thus, the aim of this work was to identify the adhesive properties of different *C. difficile* ribotypes (027, 133, 135, 014, 012) towards the ECM laminin-1 (LMN-1). The adhesion assay was performed with a direct binding assay by using coverslips coated with LMN-1. Initially, a binding test by growing the bacteria in a BHI-PRAS showed no adhesion to LMN-1, hence an inoculum in media containing different glucose concentrations (0.2, 0.5 and 1%) was carried out. Cells were counted with a LIVE/DEAD bacterial kit and all images acquired were transferred to ImageJ software for quantification. The recognition to LMN-1 was stronger for RT012 and RT027 in all glucose concentrations, while with the RT135 the adherence occurred only with 0.5% glucose, suggesting the involvement of a polymeric matrix, such as biofilm. Consequently, the biofilm production was evaluated by a method based on the quantification with crystal violet, followed by scanning electron microscope observations. All ribotypes were categorized as strong biofilm producers, but we could not correlate the biofilm with the adhesion to LMN-1. An immunolabelling by using transmission electron and fluorescence microscopy showed that the LMN-1 recognition molecule is located in the bacterial cell surface. Experiments are being conducted to identify the nature of this surface molecule by mass spectrometry. If so, mutants will be constructed to prove the involvement of this molecule in the recognition of LMN-1. We truly believe that by identifying this adhesion molecule and its cognate receptors might contribute to elucidation of *C. difficile* colonization and inflammatory disease.

# MACROPHAGE MIGRATION INHIBITORY FACTOR AGGRAVATES *CLOSTRIDIUM DIFFICILE* INFECTION IN MICE

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*Clostridium difficile* is the most important cause of nosocomial infectious diarrhea in the western world. Toxins (A, B and binary toxins) generated by *C. difficile* bacteria induce damage to intestinal epithelial cells. Ensuing inflammation of colonic mucosa including upregulation of inflammatory mediators and infiltration of inflammatory cells is one of the hallmark of the *C. difficile* infection (CDI). Macrophage migration inhibitory factor (MIF) is one of the numerous inflammatory mediator that is upregulated in CDI. MIF is known to enhance colonic inflammation in animal models of dextran sodium sulfate colitis, and MIF-depletion (MIF knockout mice or antibody-mediated MIF depletion) significantly reduces colitis.

To understand the role of MIF in CDI pathogenesis, we studied a cohort of hospitalized patients and a murine model of CDI. We found that patients with CDI have significantly high circulating MIF compared to patients who have diarrhea but tested negative for *C. difficile* (non-CDI controls). Similarly, *C. difficile* challenge significantly increased levels of tissue and plasma MIF in mice. In our mouse model, antibody-mediated depletion of MIF decreased CDI-induced diarrhea, weight loss and mortality. In addition, neutralization of MIF resulted in substantial reduction of systemic leukocytosis and colonic tissue damage after CDI.

Since CDI disease severity is dependent on intensity of host inflammation, our data suggests that blocking MIF-mediated inflammatory responses can be beneficial in CDI and improve disease outcomes. Future studies would focus on determining the impact of anti-MIF intervention on *C. difficile* pathogen and to further elucidate mechanisms that confer reduced inflammation.

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## MISPROCESSING OF CspBA BY YabG IN *CLOSTRIDIODES DIFFICILE* SPORE LOSES NECESSITY OF AMINO ACID FOR GERMINATION

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Due to the strict anaerobic nature of the vegetative form, *Clostridiodes difficile* cannot survive for extended periods of time in the aerobic environment outside a host. Thus, the spore form is important for transmission between hosts. Once in a host, spores must germinate to the vegetative form in order to cause disease. *In vitro C. difficile* spore germination is triggered by certain bile acids (e.g., taurocholic acid [TA]) and certain amino acids (e.g., glycine). In prior work, the bile acid germinant receptor was identified as CspC protein but the receptor with which glycine interacts is unclear. To identify the glycine germinant receptor, we used a chemical mutagenesis approach to introduce random mutations into the genome of *C. difficile* vegetative cells and these cells were allowed to form spores. Subsequently, spores were germinated in the presence of TA and betaine (a glycine analog that normally is not a co-germinant). We predicted that any spore that germinated in the presence of TA and betaine could have a mutation in the glycine receptor allowing it to recognize betaine as a co-germinant. Interestingly, the most frequent phenotype isolated were spores that germinated in presence of TA alone without the requirement of glycine. Sequencing several isolated mutants from several mutageneses, revealed mutations in *yabG.* YabG is a protease that is thought to cleave sporulation-specific proteins such as preproSleC and CspBA. We hypothesize that the misprocessing of CspBA in the *yabG* mutant results in spores that no longer require amino acids as co-germinants. Experiments are ongoing to test this hypothesis.

# PERSISTENCE AND MULTIPLICATION OF *CLOSTRIDIOIDES DIFFICILE* IN *ACANTHAMOEBA CASTELLANII* UNDER ANAEROBIC CONDITIONS

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*Clostridioides difficile* is the major etiological agent of the pseudomembranous colitis and diarrhea associated to antibiotics use. In recent years, with the appearance of epidemic strains, with high morbidity and mortality rates of CDI in the hospital, has raised the possibility of other sources of isolation of *C. difficile*. *Acanthamoeba castellanii* is a free-living amoeba (FLA), a ubiquitous environmental protist that contribute to the microbiological contamination and control of microbial populations in water sources, due to its predatory behavior. However, some microorganisms have developed resistance to the intracellular mechanisms of amoebas (ARMs) that benefit from protection from conditions occurring outside the protozoan host and be transmitted to an infected human host by amoeba. Thus, this study aims to investigate whether *C. difficile* can survive and multiplicate in ARMs and if its virulence profile is changed, when compared to a control not phagocytosed by *A. castellanii*, by using mass spectrometry (Orbitrap). A co-culture test between *A. castellanii* and *C. difficile* was performed to determine the amoebae's ability to engulf the bacteria in anaerobic conditions and the bacterial survival capacity through CFU/mL determination in BHI-PRAS (0.02% sodium taurocholate). All supernatants were stored at -80ºC for mass spec analysis. To determine the nature of the vacuole where the bacteria is found, and if *C. difficile* is sporulating, transmission electron microscopy (MET) images are being conducted. So far, we demonstrated the survival of *C. difficile* inside the amoeba for 1-4 h and 18 hours of incubation. Apparently, no changes occurred in either the morphology of the amoeba or to the bacteria. Thus, our study can show if *Acanthamoeba* spp. can be a source of dissemination of *C. difficile* and if the proteins expression involved in its virulence is changed.

*PII-35*

## ANTIMICROBIAL ACTION OF ENTEROCIN P AGAINST STRAINS OF *CLOSTRIDIOIDES DIFFICILE*

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The *Clostridioides difficile* infection (CDI) has a broad spectrum of symptoms, such as, pseudomembranous colitis and toxic megacolon. CDI is closely related to the use of antibiotics, therefore, new alternatives to classical antibiotics are of great importance in the treatment and prevention of CDI. One of the alternatives is the use of enterocins (antimicrobial peptides or proteins being ribosomally synthesized), for instance, bacteriocins produced by the genus Enterococcus that classically present inhibitory activity against gram-positive bacteria. Thus, the present work aims to evaluate the antimicrobial activity of enterocin P, produced by *Enterococcus faecium* E86 strain, against different isolates of commensal gut bacteria and pathogenic *C. difficile* ribotypes. To the assay, a solid phase co-culture methodology, based in dot-on-plate inoculation followed by an incubation under anaerobic conditions for 24 h, was used. As a negative control, the ATCC 10100 *Enterococcus faecalis* strain, which does not present antibacterial activity, was also included in the assay. The confirmation of the inhibitory activity was analysed by observing the presence of inhibition halos around the bacteriocinogenic strain. Our preliminary results demonstrated that enterocin P affected the the growth of at least eight *C. difficile* ribotypes, including the epidemic strain BI/NAP1/027. Conversely, the same was not observed to *Clostridium citroniae, Clostridium scindens* strains and species of the genus *Lactobacillus* belonging to intestinal microbiome. For further analysis, a co-culture in broth medium will be performed, following UFC counting, to estabilish the minimal inhibitory concentration of the sensitive strains. Transmission electron microscopy images will evaluate how the enterocin affects the cell structure of *C. difficile*. Initial data show that the action of enterocin P on strains of *C. difficile* needs to be elucidated since it represents a possible tool against antibiotic-resistant strains found in the clinic in the next years.

# INCIDENCE AND MOLECULAR CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* ISOLATED FROM ANIMALS, AND THE ASSOCIATED CHANGES IN THE HOST FECAL MICROBIOTA

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The relationship between the gut microbiota and *Clostridium difficile*, and its role in the severity of *C. difficile* infection in humans is an area of active research. Intestinal carriage of toxigenic and non-toxigenic *C. difficile* strains, with and without clinical signs, is reported in animals, however very few studies have looked at the role of the host gut microbiota. This study aims to define the incidence rate of *C. difficile* from different animal species. In addition, *C. difficile* strains isolated from animals were characterized by their toxin gene profile and ribotype. The feces from animals shedding *C. difficile* were used in a Vero cell cytotoxicity assay, and all samples were analyzed by 16S rRNA gene sequencing. Preliminary data on 50 fecal samples from six different host species including dogs (28), cats (4), horses (11), goats (5), alpaca (1), and chicken (1) were collected. *C. difficile* was recovered from 20% of samples (10/50), which included 8 dogs, 1 cat, and 1 horse. All other hosts tested negative. Five plex PCR revealed two different toxin gene profiles: 8 *tcdA+*, *tcdB+*, *cdtAB-* and 2 *tcdA+*, *tcdB-*, *cdtAB-*. Overall 6 different ribotypes were identified from 10 positive isolates, and some were shared between species. The fecal microbial community structures were different between species. Microbial diversity was lower in animals that were positive for *C. difficile,* compared to negative, within the same species. *C. difficile* strains isolated from animals shared similar toxin gene profiles, and ribotypes compared to human cases. A One Health approach with a larger cohort of animals is needed to better understand the dynamics between *C. difficile* and the gut microbiota.


JULY 9-12, 2018
ANAEROBE 2018
Las Vegas, Nevada

***1300 POSTER SESSION III: CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY***



Posters will be presented in Poster Session II
Thursday, July 12 1300-1400.

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## TOXIGENIC *CLOSTRIDIUM DIFFICILE* ARE HIGHLY PREVALENT IN COMMUNITY SHOE SWABS

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**Background:** Environmental surfaces can be frequently contaminated with animal and bird fecal materials in any geographical location. Spores of *C. difficile* can survive for months on fecally contaminated surfaces. Shoe bottoms are in frequent contact with these contaminated materials and thus, can be contaminated frequently with *C. difficile* spores in any geographical areas as seen in our Houston, Texas community studies. To investigate shoes as source of *C. difficile* contamination on a worldwide basis, we collected shoe bottom swab samples from two unique geographic locations and cultured to isolate and characterize the *C. difficile* isolates.

**Method:** As part of a hospital-wide surveillance effort, we collected shoe-bottom swabs samples from Mumbai, India (n=187) and Nuevo Laredo, Mexico (n=65). Samples were assessed for *C. difficile* using anaerobic enrichment culture and molecular methods. Suspected colonies from cycloserine cefoxitin fructose agar (CCFA) plates were identified and characterize by PCR (*tpi, tcdA, tcdB, cdtA, cdtB*) and strain typed using fluorescent PCR ribotyping.

**Result:** A total 101 of 187 (54.0%) shoe-bottom swab samples from India, and 22 of 65 (33.8%) from Mexico were culture positive for *C. difficile* of which 36.4% (India) and 16.9% (Mexico) samples were toxigenic (*tcdA* and *tcdB*) *C. difficile*. A total of 20 distinct ribotypes were identified from 72 *C. difficil*e isolates tested from India. A total of 11 distinct ribotypes were identified from 20 isolates from Mexico. Predominant ribotypes were F106, F014-020, F005, and F002.

**Conclusion:** We have found a high prevalence of toxigenic *C. difficile* with diverse ribotypes from shoe bottom swabs in two geographical locations and such pathogenic strains can be the source of human- or animal-gut colonization/infection. Results are consistent with Houston, Texas area data.

# COMMUNITY-ACQUIRED INFECTION WITH HYPERVIRULENT *CLOSTRIDIUM DIFFICILE* ISOLATES THAT CARRY DIFFERENT TOXIN AND ANTIBIOTIC RESISTANCE LOCI: A CASE REPORT

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*Clostridium difficile* infection (CDI) leads to the onset of antibiotic-associated diarrhea (AAD) and a wide range of gastrointestinal pathologies. Currently, CDI is one of the most important opportunistic infections at the intrahospital level and an exponential increase in community-acquired infections has been reported. Herein, we evaluated the relationships (at phylogenetic and genetic population structure levels), as well as the molecular toxigenic and antibiotic resistance profiles of a set of isolates established from a case of community acquired-CDI.

**Case presentation:** A 30-year-old woman with no history of hospitalization who was exposed to antibiotics (ampicillin/sulbactam and metronidazole) after a cat-bite wound was presented. The patient had a continuous episode of diarrhea; a stool sample was then collected and community acquired-CDI was confirmed by molecular tests and *in vitro* culture. Seven isolates were established and subsequently subjected to: (i) Multilocus sequence typing, all isolates belonging to ST-1 (associated with hypervirulent strain (027/BI/NAP1); (ii) description of their toxigenic profile: two of the isolates (Gcol.49 and Gcol.91) were positive for the genes coding for the major toxins (*tcdA* and *tcdB*) and their negative regulator (*tcdC*). All isolates were positive for the *cdtB* gene encoding one of the binary toxin subunits, while only two (Gcol.51 and Gcol.52) were positive for *cdtA*; and (iii) identification of antibiotic resistance molecular markers, where there was no difference in *gyrA* or *gyrB* gene polymorphisms (related to quinolone resistance), but rather at loci presence/absence, being just one isolate negative, whereas the others showed a differential presence of the *tet*, *ermB* and Tn916 regions. The former was associated with resistance to tetracycline and the other two for erythromycin/clindamycin. In conclusion, this case represents the first report of community acquired-CDI in Colombia associated with hypervirulent strains and shows that isolates obtained from a single patient can carry different toxin and antibiotic resistance loci.

*PII-39*

## LACK OF IMPACT OF AGE ON *CLOSTRIDIUM DIFFICILE* INFECTION (CDI) RISK AMONG MEDICARE RECIPIENTS

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**Background:** Predictive models can be used to identify patients at higher risk of CDI in order to target prevention activities. We compared the performance of multivariable models including sets of variables to determine the impact of underlying acute and chronic conditions, infections, healthcare utilization, and age on CDI prediction in an elderly U.S. population.

**Methods:** Persons aged 66 years and older coded for CDI in 2011 were identified using Medicare claims data; the comparison population consisted of uninfected persons in the 5% random sample. Potential risk factors for CDI were identified and organized into these categories: age, chronic comorbidities (CC), frailty indicators (FI), acute noninfectious conditions (AC), infections and healthcare utilization. Multivariable logistic regression with CDI as the dependent variable was performed to determine the impact of removing individual categories on model performance, defined by the change in the Bayesian Information Criterion (BIC), deviance, and the C statistic.

**Results:** 174,903 persons coded for CDI and 1,453,867 uninfected persons were included. The full model had 87 predictor variables and C statistic=0.918. Compared to the full model, removal of age had no impact on the C statistic (C statistic=0.918) and the BIC improved (ΔBIC=-20). Removal of CC, FI, and AC, respectively, from the full model also resulted in minimal change in the C statistic (0.917 for CC and FI, 0.916 for AC); ΔBIC=3,948, 4,969, 6,877, respectively. The biggest change compared to the full model was associated with removal of healthcare utilization (C statistic 0.897, ΔBIC=63,894) followed by acute infections (C statistic=0.911, ΔBIC=25,328). Only these two models performed significantly worse than the full model by the deviance statistic.

**Conclusions:** Inclusion of age in a comprehensive model to predict CDI in elderly persons had no impact on model performance. These results suggest that increasing age does not contribute to prediction of CDI in the elderly population, after taking into account other important conditions, infections, and healthcare encounters.

# GENOMICS OF *CLOSTRIDIOIDES DIFFICILE* REVEAL DISEASE TRANSMISSION BETWEEN FACILITIES WITHIN A SINGLE HOSPITAL NETWORK

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Isolates of *Clostridioides* (formerly *Clostridium*) *difficile* that are Sequence Type 1 (ST1: i.e. NAP1 and/or ribotype 027) are hypervirulent strains associated with nosocomial infections that cause considerable morbidity and mortality. The purpose of our study was to genomically characterize ST1 *C. difficile* isolates from a single healthcare network in Arizona (USA) and identify disease transmission patterns to aid infection prevention. We purified 26 isolates under anaerobic conditions from patient diarrheal samples collected from Mar. 2016 through Sept. 2017. Genomic DNA was extracted and sequenced using the Illumina MiSeq platform. Sequences were compared at the single-nucleotide level (>3.8Mbp compared) using NASP and differences (SNPs; single-nucleotide polymorphisms) were used to determine transmission events and construct a phylogenetic tree. Results from a LS-BSR analysis comparing the presence/absence of all coding regions were also mapped to the phylogenetic tree. Using previously established bounds for determining transmission events in *C. difficile* based on SNPs, we found that five of our 26 isolates were genetically distinct. The remaining 21 isolates formed four potential transmission clusters and within these clusters multiple isolates had ≤2 SNPs indicating direct transmissions. Examination of hospital contact trace data for one of these cases indicate transmission somehow occurred between patients residing at different facilities; this transmission event would not have been discovered using traditional, hospital contact tracing alone. Additionally, large numbers of SNPs (>40) and differing coding region profiles identified during our analysis also refuted other suspected transmission events identified by the hospital. Our study highlights the utility of genomics in a hospital setting and revealed that the scope of infection prevention for *C. difficile* disease must be expanded from a single hospital unit or facility to the entirety of the hospital network.

*PII-41*

## MOLECULAR EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE* ISOLATED IN THE UNITED STATES, 2016

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Both the incidence and severity of *Clostridium difficile* infections (CDI) increased dramatically with the emergence of the epidemic ribotype (RT) 027. While there have been only modest declines in the incidence of CDI in the United States, CDI severity and incidence has declined markedly in England over the past ten years, associated with a decline in RT 027 frequency that has been attributed to decreased fluoroquinolone prescribing. In 2009, the Centers for Disease Control and Prevention (CDC) implemented CDI surveillance through the Emerging Infections Program (EIP) to monitor the rate and evolving epidemiology of CDI. Here we describe the molecular epidemiology of *Clostridium difficile* isolates collected in the United States in 2016.

In 2016, CDI surveillance was conducted at 10 EIP sites (CA, CO, CT, GA, MD, MN, NM, NY, OR, and TN). A convenience sample of clinical laboratories across EIP sites submitted *C. difficile*-positive stool specimens to the MN Department of Health Public Health Laboratory and Hines VA Hospital for culture. Isolates were forwarded to CDC and characterized by capillary-based PCR-ribotyping and PCR detection of *tcdA*, *tcdB*, *cdtA*, *cdtB*, and deletions in *tcdC*.

Among 962 *C. difficile* isolates submitted in 2016, 137 unique RTs were observed. The majority of isolates were positive for toxin genes *tcdA* and *tcdB* (95%) and had a wildtype *tcdC* sequence (68%). The five most common RTs observed among both healthcare- (HA) and community-associated (CA) isolates were RTs 027, 106, 014, 002, and 020, which accounted for 45% and 40% of 2016 submissions, respectively. Among 503 HA isolates, RT 027 was most prevalent and predominated at 5 EIP sites (CO, CT, GA, OR, TN). The overall frequency of HA RT 027 did not change significantly from 19% in 2015 to 15% in 2016 ($p=0.22$). Meanwhile, RT 106 was most prevalent among 459 CA isolates and predominated at 7 sites (CA, CO, CT, MD, MN, NY, TN); RT 106 increased among CA cases from 9% in 2015 to 14% in 2016 ($p=0.063$).

In contrast to England, RT 027 in the United States remained the leading cause of HA CDI in 2016 and did not change significantly from the previous year, suggesting the implementation of additional HA CDI-tailored prevention strategies are needed. Meanwhile, increased understanding of strain dynamics in the epidemiology of CA CDI is needed.

# STUDY OF TOXIGENIC *CLOSTRIDIUM DIFFICILE* AND ITS ANTIBIOTIC SUSCEPTIBILITY PROFILE FROM CHICKEN DROPPINGS IN NIGERIA

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The prophylactic usage of antibiotics to prevent poultry diseases has overtime been abused and in most cases has led to *Clostridium difficile* infection (CDI) which causes 10%-30% of antibiotic associated diarrhoea. In Nigeria, the rate of poultry and poultry products consumption is high as it contributes largely to her economy and to our knowledge, little or no studies on *C. difficile* infection in poultry has been carried out. Also, there are no stringent government regulations guiding antibiotics prescriptions for humans and animals thus the epidemiological study of *C. difficile* is important. In this study, a total of 264 poultry feacal samples which included broilers (n=113), layers (n=99) and chicks (n=52) were collected from five different farms between July and September, 2017 and analysed for their prevalence and antibacterial susceptibility. The result revealed that 16% (n=42) were *C. difficile* positive with Oke-Aro farm having the highest prevalence 33% (n=13) and Obasanjo farms with lowest prevalence 9.9% (n=9). The prevalence in other farms ranged between 17-12% and was found to be statistically significant ($p=0.022$, $\alpha = 0.05$). Out of the 42 isolates, 31(73.8%) were positive for toxin production while 11 (26.2%) were non-toxigenic. A total of 13 (31%) chickens produced both toxin A and B while 11(26.2%) and 7 (16.7%) tested positive for toxin A and toxin B respectively. Antibacterial susceptibility testing revealed a growing resistance to Sulphamethoxazole (69%), Cloxacillin (64.3%) and Quinolone (7.41%). The isolation of *C. difficile* from the different chickens could be attributed to the differences in the sanitary and level of bio-security measures used in the different farms. Although there has been no reported outbreak of *C. difficile* infection in all the farms sampled, the possibility of an outbreak in the long run is imminent. Therefore, indiscriminate addition of antibiotics in poultry management should be cautioned. It is anticipated that the results of this research will enable the federal government to develop relevant stringent policies towards controlling/regulating antibiotic usage in poultry farms in Nigeria.


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***1300*** ***POSTER SESSION II: CLOSTRIDIUM DIFFICILE:* MANAGEMENT**



Posters will be presented in Poster Session II
Thursday, July 12 1300-1400.

*PII-43*

## FITNESS COSTS OF *CLOSTRIDIUM DIFFICILE* RIDINILAZOLE REDUCED SUSCEPTIBILITY MUTANTS

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Ridinilazole is a novel antibiotic currently initiating phase III studies for the treatment of *Clostridium difficile* infection. To better understand the mechanism of action of ridinilazole a mutant isolate with reduced susceptibility was developed. The purpose of this study was to assess fitness costs associated with the mutant isolate vs. wild-type. *C. difficile* strains 630 and R20291 were subjected to 15 passages in BHIS supplemented with ridinilazole at multiple concentrations. Bacterial populations growing at >8xMIC values for three passages were subjected to 3 extra passages in BHIS only and then re-exposed to the specific antibiotic. Control and mutant strains were grown in BHIS supplemented with oxyrase for 24h in 24 well plates to measure optical density at 600nm with 1h intervals. Toxin levels were quantified via ELISA at 24 and 48h. A phenotypic analysis was also made on these specific mutants by scanning electron microscopy.

No stable mutants were observed with the 630 strain exposed to any of the antibiotics tested. The R20291 mutants exposed to ridinilazole were stable at 8xMIC (0.5mg/L) after 3 passages without antibiotic pressure. Ridinilazole mutant strains displayed a stable filamentous phenotype with markedly increased cell length (greater than 100µm) similar to susceptible cells treated with sub-MIC ridinilazole concentrations. Growth curves of the mutants presented a highest slope coefficient during the exponential phase and were lacking the plateau phase compared to the wild type. 24h toxin levels (A and B) were similar to control, results show a decrease at 48h in mutants compared to wild type.

Resistance development against ridinilazole occurred in *C. difficile* strain R20291 with a phenotype similar to the one observed after treatment at low concentrations of ridinilazole. Evidence suggests a fitness cost to *C. difficile* isolates with reduced ridinilazole susceptibility.

# METRONIDAZOLE RESISTANCE MECHANISMS IN *CLOSTRIDIUM DIFFICILE*

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**Background:** Metronidazole (MTZ) has been a mainstay drug for *C. difficile* infections (CDI), but treatment failure is now common and reasons are unclear. MTZ resistance (MTZ-R) is prevalent, especially in epidemic 027 strains. We resolved issues for proper detection for MTZ-R, showing heme is a critical factor. Herein, we elucidated genetic mechanisms of MTZ-R in *C. difficile*.

**Methods:** MTZ resistant *C. difficile* were analyzed from: (**1**) a non-toxigenic mutator of ATCC700057 on MTZ agars; (**2**) *in vivo* mutants from hamsters treated with MTZ for CDI; and (**3**) patient isolates. After genome sequencing, key mutations were confirmed by Sanger sequencing and validated by gene deletion, knockdown and complementation strategies.

**Results:** Since mutants could not be attained from WT, we subjected the mutator to passaging, showing attainment of changes to iron transporter (FeoB, *feoB*), oxidoreductases (PFOR, *nifJ*) and iron-sulfur regulator (IscR, *iscR*). Mutations in *feoB* appeared to be the first step towards resistance. Hence deletion of *feoB* in WT, in combination with CRISPR-dCas9 knockdown of *iscR* or *nifJ* conferred resistance (MIC=8 mg/L) versus the WT (MIC=0.25 mg/L). This suggests epistasis between these iron-regulated processes promotes MTZ-R. Serial passaging of Δ*feoB* strain led to MTZ-R evolution, showing loss of this gene provides a gateway for resistance. *In vivo* mutants (MIC=8-16 mg/L) carried mutations in peroxide stress regulator (*perR)*, which upon complementation with the WT gene reversed the phenotype. Clinical isolates had mutations in genes predictive of MTZ-R, such as *fhuB/feoB* (iron transporters), *nifJ*, *rbr* (rubrerythrin) and *etfB* (electron transfer flavoprotein B).

**Conclusions**: Gene disruption patterns in lab-derived mutants and clinical isolates converged, providing new insights to MTZ-R pathoevolution in *C. difficile*. This study is urgently needed to understand why MTZ is faltering in the clinic and to promote adherence to the 2018 IDSA/SHEA CDI guidelines.

*PII-45*

## BEZLOTOXUMAB FOR PREVENTION OF *CLOSTRIDIUM DIFFICILE* INFECTION RECURRENCE: DISTINGUISHING RELAPSE FROM REINFECTION WITH WHOLE GENOME SEQUENCING

Dorr, M.B.;*[1] Zeng, Z.;[1] Wilcox, M.H.;[2] Li, J.;[3] Poxton, I.;[4] Zhao, H.;[3] Li, H.;[3] Guris, D.;[1] Shaw, P.[1]
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**Background:** Bezlotoxumab (BEZ) and actoxumab (ACT) are monoclonal antibodies against *C. difficile* toxins B and A, respectively. Patients receiving a single infusion of BEZ alone or with ACT in the MODIFY I/II trials showed an absolute 10% (relative ~40%) reduction in rCDI over 12-weeks compared with placebo (PBO). Addition of ACT did not improve efficacy. This *post hoc* analysis investigated whether BEZ prevented relapse with the same strain and/or reinfection with a new strain.

**Methods & Results:** *C. difficile* strains isolated from patient stool samples were typed by PCR ribotyping, PCR free library construction and Illumina whole genome sequencing (WGS). rCDI was defined as diarrhea with toxigenic *C. difficile* in stool. Reinfection and relapse were differentiated by comparing ribotype (RT) and pair-wise single-nucleotide WGS variations (PWSNV). Relapse was assigned if the baseline RT and the RT isolated during rCDI were the same and PWSNVs were ≤2. Reinfection was defined as rCDI cases with a different RT compared with baseline or the same RT with >10 PWSNVs. Patients receiving BEZ or ACT+BEZ were pooled and patients receiving PBO or ACT were pooled. BEZ effect on cumulative incidence of relapse and reinfection was estimated by Fine & Gray's competing risks survival model. Of 514 patients with rCDI, 259 (50.4%) had baseline and post-baseline *C. difficile* isolates. There were 198 (76.4%) relapse and 50 (19.3%) reinfection cases. Among rCDI cases, proportions of reinfection and relapse were similar between treatments. Proportion of relapses was higher for RT 027. Significant differences in crude cumulative incidence for relapse ($p<0.001$) were observed for BEZ and ACT+BEZ groups compared with PBO and ACT groups. Similar changes were observed for reinfection but results were not significant. Cumulative incidence curves showed that relapses occurred earlier and at a higher rate than reinfections, but the reduction in rCDI was similar.

**Conclusion**: BEZ-induced reduction in rCDI reflects the prevention of relapses. Reinfection reduction was also observed, but likely due to a smaller number of reinfection cases, the difference was not significant.

# A LYOPHILIZED, NON-FROZEN, ORAL MICROBIOTA-BASED DRUG RBX7455 IS SAFE, REDUCES *CLOSTRIDIUM DIFFICILE* INFECTION RECURRENCE, AND RESTORES GUT MICROBIOME

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**Purpose:** To broaden access to microbiota-based therapeutics therapies, RBX7455—a lyophilized, non-frozen, orally-administered microbiota-restoring drug candidate was developed. We report interim results from an open-label Phase 1 trial of RBX7455 for preventing recurrent *Clostridium difficile* infections (rCDI).

**Methods:** Nineteen patients with ≥2 CDI episodes following ≥2 courses of antibiotic therapy were enrolled. Patients received 8 RBX7455 capsules for 4 days (cohort 1) or 2 days (cohort 2). Success was defined as absence of CDI recurrence through 8 weeks after treatment completion, and adverse events were monitored during and after treatment.

Patients stool samples (prior to and at time points after treatment) and representative RBX7455 samples were sequenced using an ultra-shallow shotgun sequencing method. Operational taxonomic unit (OTU) data were grouped by cohort and compared using a Bray-Curtis dissimilarity calculation. Relative OTU abundances at the class level were compared among time points.

**Results:** Nine of ten patients in cohort 1 (median age=67, 90% female) and seven of nine patients in cohort 2 (median age=54, 55% female) were recurrence-free at the 8-week endpoint, for an overall efficacy of 87%. A total of 37 non-serious adverse events (AE) were observed, with gastrointestinal AEs being most common. There were no serious AEs observed.

Prior to treatment, the taxonomic compositions of responder microbiomes were dissimilar from the RBX7455 composition and were dominated by Gammaproteobacteria and Bacilli. After treatment, patient microbiomes converged toward the RBX7455 composition, with Bacteroidia and Clostridia becoming more predominant. Microbiome changes were comparable among responders in both cohorts.

**Conclusion:** Across two dosing regimens, RBX7455 had 90% and 78% success in preventing rCDI with no serious AEs. In addition, RBX7455 appears to restore patient microbiomes toward the RBX7455 composition. Microbiome and safety data collection will continue to 6 months after treatment.

This analysis was funded by Rebiotix Inc., Roseville, MN.

*PII-47*

## *CLOSTRIDIUM DIFFICILE*: ANTIBIOTIC SUSCEPTIBILITY AND $MLS_B$ RESISTANCE MECHANISM

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The purpose of this study was to detect virulence factors and antibiotic profile of *Clostridium difficile* strains isolated in Silesia, Poland: 164 strains isolated from fecal samples of hospitalized patients suspected for CDI were studied. In fecal samples presence of GDH antigen and *C. difficile* A/B toxins were studied by membrane, EIA and Illumigene® *C. difficile* tests. Fecal samples were cultured, suspected colonies were identified using VITEK 2 Compact system and antibiotic susceptibility to 11 antibiotics was tested by EUCAST recommendation. Genes encoded GDH and toxins (A, B, binary) were detected by mPCR and *ermB* gene was detected by appropriate PCR.

In all studied *C. difficile* strains presence of *gluD* gene was confirmed. Gene *ermB* was detected in 45 strains, in 42 of them genes encoding toxins A, B and binary toxin were detected; in 1 strain all toxin genes were absent, in 2 remaining strains only toxin B gene was absent, and in 1 of them only binary toxin gene was confirmed.

In remaining 119 strains without *ermB* gene in 97 all toxin genes were confirmed, in 4 strains - all of them were absent, in 2 strains only toxin A and binary toxin genes were detected, in 16 - only toxin A gene was present. All strains were sensitive to metronidazole, vancomycin, amoxicillin/clavulanic acid and piperacillin/tazobactam. *ermB* gene was present in 33 strains among 139 resistant to erythromycin and 72 - to clindamycin. Geometric means (GM) to erythtomycin and clindamycin of all studied strains were 84.28 i 14.39, respectively. However, in *ermB* positive strains GMs were significantly higher, than in *ermB* negatives, respectively 181,26 vs. 80,86 for erythtomycin and 107,67 vs. 2,8 for clindamycin. Resistant to ciprofloxacin were 164 strains, to moxifloxacin – 135. GMs to moxifloxacin and ciprofloxacin were 13.82 and 28.01; in *ermB*-positives and negatives, respectively for moxifloxacin 24.63 vs. 13.6 and for ciprofloxacin 31.03 vs. 28.61.

Among studied strains 100 were resistant to penicillin G, 124 – to imipenem, 52 – to rifampicin.

MDR was confirmed in 26 (15.9%) strains, in 23 of them *ermB* gene was present.

## CIRCULATING INFLAMMATORY MEDIATORS IN THE SETTING OF SEVERE *CLOSTRIDIUM DIFFICILE* INFECTION

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Accurate biomarkers for severe *Clostridium difficile* infection (CDI) can inform medical decision-making but are currently lacking. We hypothesized that circulating inflammatory mediators would associate with severity in a prospective cohort of inpatients diagnosed with CDI (diarrhea and stool positive for toxigenic *C. difficile* by the clinical microbiology laboratory). Sera were collected upon diagnosis of CDI and frozen at −80°C until analysis. A custom, bead-based, multiplex inflammatory mediator panel was performed on samples using a Luminex 200 dual laser detection system, and all resulting measurements in pg/mL were log-transformed. Redundancy analysis (RDA), permutational MANOVA, logistic regression, and elastic net regression were used for analysis. In 275 episodes of CDI, 71 (26%) met Infectious Diseases Society of America (IDSA) severity criteria and 30-day all-cause mortality occurred in 19 (7%). RDA differentiated IDSA severe and non-severe episodes significantly by permutational MANOVA (P=.001), and the principal component axis 1 (PC1) explained 27.6% of total variance. Examining individual inflammatory mediators, the strongest associations with IDSA severity were for HGF (OR=1.98 [1.5,2.6]), procalcitonin (OR=1.57 [1.3,1.9]), IL-6 (OR=1.39 [1.2,1.7]), and IL-2R (OR=2.27 [1.5,3.5]). An elastic net predictive model selected with 10-fold cross-validation resulted in a model containing procalcitonin and hepatocyte growth factor (HGF), with an area under the receiver-operator characteristic curve (AUC) of 0.74 [0.67,0.81]. RDA also showed separation for 30-day all-cause mortality (P=.001), and PC1 explained 26.4% of total variance. The individual inflammatory mediators with the strongest associations with mortality were IL-2R (OR=8.21 [3.4,19.9]), procalcitonin (OR=1.93 [1.4,2.6]), IL-8 (OR=2.04 [1.5,2.9]), and CXCL10/IP-10 (OR=1.76 [1.3,2.4]). An elastic net model with IL-2R and PC1 had an AUC of 0.86 [0.78,0.93]. These data show that circulating biomarkers associate not only with severity of the CDI episode, but also with subsequent mortality. Future studies are needed to validate these findings and incorporate them alongside clinical variables into predictive models that could be used by clinicians.

*PII-49*

# GENOME WIDE ANALYSIS REVEALS HOST GENETIC VARIANTS ASSOCIATED WITH REDUCTION IN *CLOSTRIDIUM DIFFICILE* INFECTION RECURRENCE IN BEZLOTOXUMAB-TREATED PATIENTS

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**Background:** Bezlotoxumab (BEZ) and actoxumab (ACT) are monoclonal antibodies against *C. difficile* toxins B and A, respectively. A single infusion of BEZ alone or with ACT reduced the rate of recurrence (rCDI) compared with placebo (PBO) in patients (pts) enrolled in MODIFY I/II. Genome wide analyses were conducted to explore if host genetic variants are associated with treatment response.

**Methods & Results:** DNA was extracted from blood from pts who consented to genetic analysis (PGx population). Genetic data were generated on a commercial Axiom array platform (Affymetrix). Genotype imputation was performed using 1000 Genomes Phase 3 reference data and Impute2 software after genetic quality control. Data from BEZ and ACT+BEZ arms were combined to provide increased power. Logistic regression with likelihood ratio test was used to search for single nucleotide polymorphisms (SNPs) that strongly associate with treatment effect. SNP rs2516513 (located in the extended major histocompatibility complex (xMHC) region) with a minor allele frequency of 25% in the general population, was associated with rCDI (p=3.04E-08). rCDI rates for the PGx population and in subgroups at high/low risk for rCDI stratified by SNP rs2516513 were estimated. Carriers of the T allele of SNP rs2516513 were associated with a statistically significant reduction in rCDI in BEZ-treated pts compared to PBO-treated pts (difference 21.5%). The magnitude of the effect of the T allele on rCDI is most prominent in pts who have ≥1 risk factor for rCDI (difference 24.6%), but is also present in patients without risk factors (difference 10.6%). In CC homozygous pts, rCDI rates are similar in both treatment groups and in pts at high or low risk of rCDI.

**Conclusion:** SNP variant rs2516513 is associated with a lower rate of rCDI in pts treated with BEZ. The location of the associated genetic variant on chromosome 6 within xMHC suggests a host driven, immunological mechanism may play a role in rCDI and may predict patients most likely to respond to BEZ. As this is an exploratory finding, an independent validation study is needed.

## NOVEL MULTIVALENT VACCINES AGAINST *CLOSTRIDIUM DIFFICILE* INFECTION

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Previously, we constructed a recombinant vaccine candidate, designated mTcd138 containing the glucosyltransferase and cysteine proteinase domains of TcdB and the receptor binding domain (RBD) of TcdA. To ensure mTcd138 is atoxic, two point mutations were introduced in the glucosyltransferase domain of TcdB, which essentially eliminates mTcd138 toxicity *in vitro* and *in vivo*. The RBD of TcdB has been reported to be highly immunogenic. It was also reported that *Salmonella typhimurium* flagellin (sFliC) protected mice from death during *C. difficile* infection (CDI) by significantly delaying *C. difficile* growth in the gut. sFlic is a known potent adjuvant, and is structurally similar to *C. difficile* flagellin FliC (cFlic). To generate a vaccine candidate targeting both toxins and *C. difficile* colonization/growth, we further fused mTcd138 with the RBD of TcdB and sFliC, resulting in Tcd169Fl. Parenteral immunization of mice with Tcd169Fl induced potent immune responses against TcdA, TcdB and sFliC, and provided mice full protection infection against hyper- virulent *C.difficile* strains.


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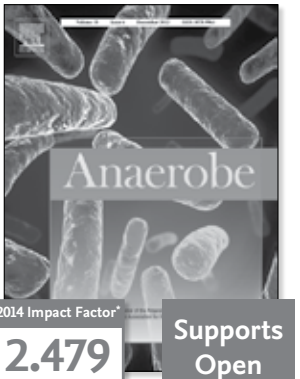


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