PROGRAM & ABSTRACT BOOK



The 13th Biennial Congress of the Anaerobe Society of the Americas

Sheraton Nashville Downtown Hotel Nashville, Tennessee



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 Carrier proteins and protein-
- Vaccine vectors suitable for nasal or oral use
- New ways to administer vaccines
- 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 and immunomodulators responses
- Biological and immunological studies
 - to further characterize adjuvants

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

Roman Chicz, PhD

Head, External Research and Development Vice President, Corporate Development Tel: +1 617 866 4562

Email: roman.chicz@sanofipasteur.com

Jean-Marc Renard, MS, MBA

Tel: +33 4 3737 3480

Email: jean-marc.renard@sanofipasteur.com



PROGRAM & ABSTRACT BOOK



Course Directors Welcome Letter iii About the Societies iv Patrons \mathbf{v} **Exhibitors** v Keynote Speaker vi Lifetime Achievement Award vii Accreditation viii Curricular Goals & Objectives viii Presenters & Faculty ix Disclosure Information xii Congress Program XV**Abstract Table of Contents** 3 Abstracts Poster Index 237 Author Index 249

The 13th Biennial Congress of the Anaerobe Society of the Americas Sheraton Nashville Downtown Hotel • Nashville, Tennessee





COURSE DIRECTOR

David M. Aronoff, M.D.ASA President
Vanderbilt University Medical Center
Nashville, TN, USA

ORGANIZING COMMITTEE

Emma Allen-Vercoe, Ph.D. University of Guelph Guelph, Canada

Karen Carroll, M.D.Johns Hopkins University
Baltimore, MD USA

Ramyavardhanee Chandrasekaran Vanderbilt University Nashville, TN USA

> **Diane M. Citron** R.M. Alden Research Lab Culver City, CA USA

Laura M. Cox, Ph.D. Harvard University Boston, MA USA

David Fredericks, M.D.ASA Secretary
University of Washington
Seattle, WA USA

Dale N. Gerding, M.D. ASA Past President Hines VA Hospital Maywood, IL USA

Ellie J.C. Goldstein, M.D. ASA Treasurer, Past President R.M. Alden Research Lab / UCLA Santa Monica, CA USA

> **Yiping Han, Ph.D.** Columbia University New York, NY USA

Purnima Kumar, D.D.S. Ohio State University Columbus, OH USA **Brandi Limbago, Ph.D.** Centers for Disease Control Atlanta, GA USA

Leandro Araujo Lobo, Ph.D. Federal University of Rio de Janeiro Rio de Janeiro, Brazil

Jeanne Marrazzo, M.D., M.P.H. ASA Vice President University of Alabama Birmingham, AL USA

Andrew B. Onderdonk, Ph.D. Harvard University Boston, MA USA

Elisabeth Nagy, M.D., Ph.D. University of Szeged Szeged, Hungary

> Carl Erik Nord, M.D. Karolinska Institute Stockholm, Sweden

Cynthia L. Sears, M.D. ASA Past President Johns Hopkins University Baltimore, MD USA

Casey J. Theriot, Ph.D. North Carolina State University Raleigh, NC

Glenn S. Tillotson, Ph.D. Cempra Pharmaceuticals Chapel Hill, NC USA

Vincent Young, M.D. University of Michigan Ann Arbor, MI USA

Ronald J. Goldman, Ph.D. ASA Executive Director Los Angeles, CA USA

ANAEROBE SOCIETY OF THE AMERICAS

PO Box 452058, Los Angeles, CA 90045 USA
Phone: 310-216-9265 Fax: 310-216-9274
Web: www.anaerobe.org E-mail: asa@anaerobe.org

©2016 Anaerobe Society of the Americas



Welcome to **ANAEROBE 2016**, the 13th 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, dialogues, and interactions 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 both traditional and emerging technologies for identification and diagnosis.

ANAEROBE 2016 again illustrates the international interest in the field of anaerobic bacteriology. 187 abstracts were submitted for presentation, representing the work of 624 scientists from more than 38 countries.

The *Keynote Address* will be given by Dr. Curtis J. Donskey of Case Western Reserve University and the Louis Stokes Cleveland VA Medical Center. Dr. Donskey will challenge traditional models of *Clostridium difficile* transmission and present new approaches to infection control.

The *Lifetime Achievement Award* will be presented to Dr. Bennett Lorber of Temple University. Dr. Lorber is being recognized for his 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 National Institutes of Health, the European Society of Clinical Microbiology and Infectious Diseases, Burroughs Welcome Fund, and the Gut Check Foundation.

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.

Lastly, I hope you have an opportunity to explore Nashville, while you are here, and savor what makes it the Music Capital of America.

Our hope is that **ANAEROBE 2016** serves to foster stimulating discussions, as well as cultivate personal relationships that continue to invigorate the entire field beyond the timeframe of this Congress.

David M. Aronoff
ASA President



About the Society

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 PRESIDENTS

This is the 13th biennial Anaerobe Society Congress. Past Anaerobe Society sponsored programs were:

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

ANAEROBE 1992—Los Angeles, CA

Patrons & Exhibitors



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
- ◆ Gut Check Foundation
- ◆ National Institute of Health

Support for this activity from commercial interests include:

PLATINUM PATRONS

- ◆ Merck
- ◆ Sanofi Pasteur

SILVER PATRONS

♦ Pfizer

Bronze Patrons

- ◆ Actelion Pharmaceuticals
- ◆ Anaerobe Journal / Elsevier
- ◆ Anaerobe Systems
- ◆ ProViotic
- ◆ Rebiotix
- ◆ Seres Therapeutics
- ◆ Summit Therapeutics
- ◆ TechLab

EXHIBITORS

- ◆ Advanced Instruments
- ◆ Bio K+ International
- ♦ bioMérieux
- ◆ Bruker Daltonic
- ◆ Cepheid
- ◆ Coy Laboratory Products

- ◆ Key Scientific Products
- ◆ List Biological Laboratories
- ◆ Microbiology International
- ◆ Shel Lab
- ◆ Synthetic Biologics
- ◆ VWR



Keynote Speaker

Lifetime Achievement Award





CURTIS J. DONSKEY, M.D.

Dr. Donskey is Professor of Medicine at Case Western Reserve University. He graduated from medical school at the Medical College of Wisconsin in Milwaukee. He completed his medical residency and chief residency at Brown University, followed by a fellowship in Infectious Diseases at Case Western Reserve University.

He has been serving as the Hospital Epidemiologist and Chair of the Infection Control Committee at the Louis Stokes Cleveland VA Medical Center since 1999.

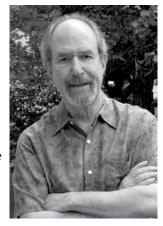
His research focuses primarily on infection control of healthcare-associated pathogens, including *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA), and multidrug-resistant gram-negative bacilli. His research group has sought to clarify the role of environmental and skin contamination in the spread of pathogens and worked to develop effective interventions to prevent transmission from these sources.

Dr. Donskey's research has been funded by the Department of Veterans Affairs, the Centers for Disease Control and Prevention, and the Agency for Healthcare Research and Quality. He has published more than 200 peer-reviewed articles and several book chapters and review articles. In addition to his research, Dr. Donskey has received awards for the teaching of medical students, residents, and fellows.

In his keynote, he will review recent evidence challenging traditional models of *C. difficile* transmission and present new approaches to reduce *C. difficile* transmission. These approaches include novel strategies to reduce the burden of spores in the environment and on skin and to prevent transmission by asymptomatic carriers. The potential impact of diagnostic testing approaches and antimicrobial stewardship on transmission will also be discussed.

BENNETT LORBER, M.D.

Dr. Lorber studied zoology and art history at Swarthmore College. He received his M.D. degree from the University of Pennsylvania School of Medicine and completed a residency in internal medicine and a fellowship in infectious diseases at Temple University Hospital, following which he was appointed to the Temple University School of Medicine faculty. In 1988, he was named the first recipient of the Thomas M. Durant Chair in Medicine at Temple. He is also a Professor of Microbiology and Immunology and served as Chief of the Section of Infectious Diseases from 1983-2006.



His interest in anaerobic infections emerged during his infectious diseases fellowship. He was responsible for a number of early studies regarding the role of anaerobic bacteria and their treatment in human respiratory, intra-abdominal, and gynecological infections. Other scholarly interests include human listeriosis, an area in which he is regarded as an international authority, along with the impact of societal changes on infectious disease patterns and the relationships between infectious agents and chronic illness. He has more than 100 publications to his credit and has contributed to every edition of *Principles and Practice of Infectious Diseases*, the authoritative textbook in the field of infectious diseases.

He is a Master of the American College of Physicians, a Fellow of the Infectious Diseases Society of America, and a Fellow of The College of Physicians of Philadelphia, where he served as President from 2010-2012. He was President of the Anaerobe Society of the Americas, 2008-2010.

He has been celebrated repeatedly for his teaching. Among his honors are 12 *Golden Apples*, the *Temple University Great Teacher Award*, the *Alexander Fleming Lifetime Achievement Award* from the Infectious Diseases Society of America, and the *Jane Desforges Distinguished Teacher Award* from the American College of Physicians. On two occasions, the graduating medical school class dedicated their yearbook to him In 1996, he received an honorary Doctor of Science degree from Swarthmore College.

In addition, Dr. Lorber is a professional painter—his work can be seen at his website: *bennettlorber.blogspot.com*—and an accomplished guitarist. He emphasizes the importance of having a hobby as a doctor. "Doing something that is important to you, makes you happy, and keeps you sane is just as important as what you do as a doctor...To best take care of patients, you have to first take care of yourself."



Accreditation/ Goals & Objectives

ANAEROBE 2016—the 13th 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 20.5 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.

Х

Presenters & Faculty

Michael Aldape, Ph.D. VA Medical Center Boise, ID USA

Emma-Allen-Vercoe, Ph.D. University of Guelph Guelph, ON Canada

David Aronoff, M.D. Vanderbilt University Nashville, TN USA

May A. Beamer University of Pittsburgh Pittsburgh, PA USA

Kathy Bernard, M.Sc. University of Manitoba Winnipeg, Canada

Karen Carroll, M.D.Johns Hopkins University Baltimore, MD USA

Robert M. Centor, M.D. University of Alabama Huntsville, AL USA

Ramyavardhanee Chandrasekaran Vanderbilt University Nashville, TN USA

Diane M. Citron R.M. Alden Research Laboratory Culver City, CA USA

Sandrine Claus, Ph.D. The University of Reading Reading, United Kingdom

Sheila Connelly, Ph.D. Synthetic Biologics, Inc. Rockville, MD USA

Georg Conrads, Ph.D. University Hospital Aachen, Germany



Laura M. Cox, Ph.D. Harvard University Boston, MA USA

Shareef M. Dabdoub, Ph.D. Ohio State University Columbus, OH USA

Charles Darkoh, Ph.D. University of Texas Houston, TX USA

Curtis J. Donskey, M.D. Louis Stokes VA Medical Center Case Western Reserve University Cleveland, OH USA

Eric Dubberke, M.D.Washington University St. Louis, MO USA

Raina N. Fichorova, M.D., Ph.D. Harvard Medical School Boston, MA USA

Sydney M. Finegold, M.D. VA Medical Center West Los Angeles, CA USA

Jane A. Foster, Ph.D. McMaster University Hamilton, ON Canada

David Fredricks, M.D.University of Washington Seattle, WA USA

Sukirth Ganesan Ohio State University Columbus, OH USA

Shiva Garg, Ph.D. Rebiotix Inc. Roseville, MN USA

χi

Caroline Attardo Genco, Ph.D. Tufts University Boston, MA USA



Presenters & Faculty

Dale Gerding, M.D. VA Medical Center Hines, IL USA

Chandrabali Ghose-Paul, Ph.D. Microbiome Medicine

New York, NY USA

Ellie J.C. Goldstein, M.D. University of California

Los Angeles, CA USA

Yiping Han, Ph.D. Columbia University

New York, NY USA

Sharon Hillier, M.D.

University of Pittsburgh Pittsburg, PA USA

Eric A. Johnson, Ph.D.

University of Wisconsin Madison, WI USA

Stuart Johnson, M.D.

Loyola University Maywood, IL USA

Colleen Kelly, M.D.

Brown University Providence, RI USA

Sarah Kuehne, Ph.D.

University of Nottingham Nottingham, United Kingdom

Ed Kuijper, M.D., Ph.D.

Leiden University

Leiden, The Netherlands

Purnima Kumar, Ph.D.

Ohio State University Columbus, OH USA

Paul Lawson, Ph.D.

University of Oklahoma Norman, OK USA

Leandro A. Lobo, Ph.D.

Federal University of Rio de Janeiro Rio de Janeiro, Brazil

Mary-Jane Lombardo, Ph.D.

Seres Therapeutics, Inc. Cambridge, MA USA

Dena Lyras, Ph.D.

Monash University Melbourne, Australia

Rajat Madan, M.D.

University of Cincinnati Cincinnati, OH USA

Michael J.G. Mallozzi, Ph.D.

University of Arizona Tucson, AZ USA

Jeanne Marrazzo, M.D., M.P.H.

University of Alabama Birmingham, AL USA

Elisabeth Nagy, M.D., Ph.D.

University of Szeged Szeged, Hungary

Mauricio Navarro, D.V.M.

University of California, Davis San Bernardino, CA USA

Maribeth Ruth Nicholson, M.D.

Vanderbilt University Nashville, TN USA

Carl Erik Nord, M.D., Ph.D.

Karolinska Institute Stockholm, Sweden

Francisca O. Nwaokorie, Ph.D.

University of Lagos Lagos, Nigeria

Andrew B. Onderdonk, Ph.D.

Harvard Medical School Boston, MA USA

Presenters & Faculty

Nisha B. Patel

University of Oklahoma Norman, OK USA

Daniel Paredes-Sabja, Ph.D.

Universidad Andrés Bello Santiago, Chile

Akshay D. Paropkari

Ohio State University Columbus, OH USA

Richard B. Pyles, Ph.D.

University of Texas Galveston, TX USA

Thomas V. Riley, Ph.D.

University of Western Australia Crawley, WA Australia

Cynthia Sears, M.D.

Johns Hopkins University Baltimore, MD USA

Anna M. Seekatz, Ph.D.

University of Michigan Ann Arbor, MI USA

Jessica Sieber, Ph.D.

University of Minnesota Duluth, MN USA

Joseph S. Solomkin, M.D.

University of Cincinnati Cincinnati, OH USA

Joseph A. Sorg, Ph.D.

Texas A&M University College Station, TX USA

Sujatha Srinivasan, Ph.D.

University of Washington Seattle, WA USA



Michael Surette, Ph.D.

McMaster University Hamilton, ON Canada

Alex G. Therien, Ph.D.

Merck & Co.

Kenilworth, NJ USA

Casey M. Theriot Ph.D.

North Carolina State University Raleigh, NC USA

Glenn S. Tillotson, Ph.D.

Cempra Pharmaceuticals Chapel Hill, NC USA

Kerin Tyrrell

R.M. Alden Research Lab Culver City, CA USA

Gayatri Vedantam, Ph.D.

University of Arizona Tucson, AZ USA

Yuanguo Wang

University of South Florida Tampa, FL USA

Mark Wilcox, M.D.

University of Leeds Leeds, United Kingdom

Vince Young, M.D.

University of Michigan Ann Arbor, MI USA

Joseph P. Zackular, Ph.D.

Vanderbilt University Nashville, TN USA

xiii xii



Disclosure Information

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

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

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

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

Disclosure Information



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.

Michael Aldape, Ph.D. Michael J.G. Mallozzi, Ph.D. May A. Beamer Elisabeth Nagy, M.D., Ph.D. Kathy Bernard, M.Sc. Mauricio Navarro, D.V.M. Robert M. Centor, M.D. Maribeth Ruth Nicholson, M.D. Ramyavardhanee Chandrasekaran Carl Erik Nord, M.D., Ph.D. Georg Conrads, Ph.D. Francisca O. Nwaokorie, Ph.D. Shareef M. Dabdoub, Ph.D. -- None Andrew B. Onderdonk, Ph.D. Charles Darkoh, Ph.D. Daniel Paredes-Sabja, Ph.D.

Raina N. Fichorova, M.D., Ph.D Akshay D. Paropkari, Ph.D. Sydney M. Finegold, M.D. Nisha B. Patel

Jane A. Foster, Ph.D.

David Fredricks, M.D.

Sukirth Ganesan

Caroline Genco, Ph.D.

Yiping W. Han, Ph.D.

Sharon Hillier, Ph.D.

Eric A. Johnson, Ph.D.

Richard B. Pyles, Ph.D.

Cynthia Sears, M.D.

Jessica Sieber Ph.D.

Joseph A. Sorg, Ph.D.

Sujatha Srinivasan, Ph.D.

Michael Surette, Ph.D.

Casey Theriot, Ph.D.

Purnima Kumar, D.D.S, Ph.D. Kerin Tyrrell

Paul Lawson, Ph.D. Gayatri Vedantam, Ph.D.

Leandro A. Lobo, Ph.D. Yuanguo Wang

Rajat Madan, M.D. Joseph P. Zackular, Ph.D.

The following presenters have information to disclose as follows:

Emma Allen-Vercoe, Ph.D. Nubiyota (O)
David M. Aronoff, M.D. Summit (C)

Karen Carroll, M.D. Abbott (G), Accelerate Diagnostics (G),

BD Diagnostics (G), Curetis (G), Quidel (C)

Diane M. Citron Anaerobe Systems (S)

Sandrine Claus, Ph.D. Novartis (G)

Sheila Connelly, Ph.D. Synthetic Biologics (E, O)
Mike Cox Anaerobe Systems (E, O)

Laura M. Cox, Ph.D. Anaerobe Systems (O), Symbiotic Health (C) Curtis J. Donskey, M.D. 3M (C), Cepheid (G), Ecolab (G), Merck (G),

Pfizer (G) Seres Health (C)

Erik R. Dubberke, M.D. Merck (C, G), Rebiotix (C, G), Sanofi

Pasteur (C, G), Summit (C)

Will discuss results of clinical trials

Shiva Garg, Ph.D. Rebiotix (E)

Dale Gerding, M.D. Actelion (C), DaVolterra (C), Merck (C), Pfizer (C),

Rebiotix (C), Seres (C), SanofiPasteur (C) Summit

(C), ViroPharma/Shire (C)



Disclosure Information

Congress Program



Chandrabali Ghose-Paul, Ph.D. Ellie J.C. Goldstein, M.D. Symbiotic Health (E)

Amicrobe (G), Astellas (G), Avidbiotics (G), Bayer Pharmaceuticals (A, S), BioK+ (A), Cerexa (G), Clinical Microbiology Institute (G), Durata (G), Forrest Pharmaceuticals (G), Genzyme (G), GlaxoSmith Kline (G), GLSynthesis (G), Gynuity Health Projects (G), Immunome (G), Impex

Pharmaceuticals (G), Kindred Healthcare (A), Merck & Co (A, G, S), Nanopacific Holdings Inc. (G), Novartis (A, G), Pfizer (G), Rempex (A, G) Romark Laboratories (G), Sankyo-Daichi (A), Salix (G), Sanofi-Adventis (A), Summit (A, G), Symbiomix Therapeutics (G), The Medicines Company (G), Theravance (G), Toltec Pharmaceuticals (G), Viroxis

(G), Warner Chilcott (G)

Stuart Johnson, M.D. BioK+ International (C), Seres Therapeutics (C),

Summit Therapeutics (C)

Will discuss Investigational Products

Colleen Kelly, M.D. Assembly Biosciences (R), Seres Health (C)

Sarah Kuehne, Ph.D. Summit (G)

Ed Kuijper, M.D., Ph.D. Astellas (C), Actelion (C), DaVolterra (C), Merck (C),

Valneva (C, G)

Mary-Jane Lombardo Seres (E, O)

SER-262 is an investigational product

Dena Lyras, Ph.D. Immuron Limited (C)

Jeanne Marrazzo, MD. Cepheid (C), Perrigo (C), Toltec (G)

Thomas V. Riley, Ph.D. Alere (R)

Anna Seekatz, Ph.D. Medimmune (G)

Joseph S. Solomkin, M.D. AstraZeneca (C) Merck (C), Pfizer (C), Tetraphase (C)

Alex G. Therien, Ph.D. Merck (E, S)

Glenn S. Tillotson, Ph.D. Cempra Pharmaceuticals (E) Summit (R)

Mark Wilcox, M.D. Abbott (G), Actelion (C,G), Alere (C,G), Astellas (C,

G), Astra Zeneca (C, G), Basilea (C), Biomerieux (G), Bayer (C), Cerexa (G), Cubist (C, G), Da Volterra (G), Durata (C), European Tissue Symposium (C, G), Johnson & Johnson (C), Merck (C,G), Nabriva (C), Novacta (C), Optimer (C), Pfizer (C, S), Qiagen (G), Roche (C), Sanofi-Pasteur (G), Seres (C), Summit (G),

The Medicines Company (G)

Vincent Young, M.D. Medimmune (G), Merck (C), Vedante (C)

C=Consultant, E-Employment, G=Grants, O=Ownership/Stock, R-Research, S=Speaker

Monday, July 11

g v

WORKSHOPS & CONGRESS REGISTRATION OPENS

P

ANAEROBIC IDENTIFICATION & SUSCEPTABLITY WORKSHOP

Diane M. Citron Mike Cox

N

EXAMINING ANAEROBES IN THE MICROBIOME: METAGENOMIC AND CULTURE APPROACHES

Laura M. Cox, Ph.D. Casey Theriot, Ph.D Anna Seekatz, Ph.D.

1800

BLUES, BREWS & BBQ AT BB KINGS 152 2nd Ave. N.





Congress Program

Tuesday, July 12

REGISTRATION / BREAKFAST / EXHIBITS

WELCOME REMARKS

David Aronoff, M.D., ASA President

SESSION I: TREATING MULTI-DRUG RESISTANT INFECTIONS IN THE ERA OF ANTIMICROBIAL STEWARDSHIP

Convener: Ellie Goldstein, M.D.

SI-1 The Clinical Presentation of *Fusobacterium necrophorum* Pharyngitis *Robert M. Centor, M.D.*

SI-2 The New Agents for Complicated Intra-Abdominal Infections: What Are the Trials Saying?

Joseph S. Solomkin, M.D.

SI-3 Parallels between *C. difficile* Colitis and Autism *Sydney M. Finegold, M.D.*

SI-4 The Non-Existent Drug Pipeline for Anaerobic Infections *Ellie J.C. Goldstein, M.D.*

945-1000 BREAK / EXHIBITS

SESSION II: KEYNOTE ADDRESS

Convener: David Aronoff, M.D.

SII-1 Never Let a Crisis Go to Waste: Progress in Prevention of Clostridium difficile Transmission Curtis J. Donskey, M.D.

SESSION III: ORAL ABSTRACTS: NON-DIFFICILE CLOSTRIDIA AND INTRA-ABDOMINAL INFECTIONS

Convener: Andrew B. Onderdonk, Ph.D.

SIII-1 Diet Fiber Contributes to Peritonitis by Activating Inflammasome Nlrp3 and Inducing IL-1ß Secretion

Leandro A. Lobo, Ph.D.

SIII-2 Detection of Toxigenic Clostridium perfringens and Clostridium botulinum from Food Commodities in Lagos State, Nigeria Francisca O. Nwaokorie, Ph.D.

SIII-3 Purification and Properties of a Novel Botulinum Neurotoxin

Eric A. Johnson, Ph.D.

SIII-4 Pathology of Bacillary Hemoglobinuria Produced by Clostridium haemolyticum

Mauricio Navarro, D.V.M.

SIII-5 The Comparative Efficacy of Antibiotics against Experimental

Clostridium septicum Infection Michael Aldape, Ph.D.

Congress Program



LUNCH / EXHIBITS 1200-1315 STUDENT COMPETITION PRESENTATIONS 1315-1415 POSTER SESSION I / EXHIBITS SESSION IV: DEFINING THE FUNCTION OF THE GUT USING OMIC APPROACHES FOR RATIONAL DESIGN OF PERSONALIZED THERAPEUTICS Convener: Casey M. Theriot, Ph.D. SIV-1 Rational Design of Microbiota-Mediated Secondary Bile Acids in the Gut to Restore Colonization Resistance against C. difficile Casey M. Theriot, Ph.D. SIV-2 Nutrimetabonomics to Understand Host-Pathogen Response to Antibiotic Treatment Sandrine Claus, Ph.D. SIV-3 Methanogens in the Gut and their Interactions with Beneficial **Butyrate Producers** Jessica Sieber, Ph.D. 1530-1545 BREAK / EXHIBITS SESSION V: LITERATURE REVIEW IN ANAEROBIC SCIENCES SV-1 An Update on Fusobacterium Disease Pathogenesis Cynthia Sears, M.D. SV-2 Bacteroides: Beyond the Microbiome Glenn S. Tillotson, Ph.D. SESSION VI: ORAL ABSTRACTS: POTPOURRI Convener: David Fredricks, M.D. SVI-1 Taking the Next Giant Step: Designed Microbiome Therapeutics for Clostridium difficile Infection (CDI) Mary-Jane Lombardo, Ph.D. SVI-2 'In Silico' Chemotaxonomy: A Tool for Microbial Systematics Nisha B. Patel SVI-3 First Genome Sequence of the Opportunistic Pathogen Clostridium septicum Michael J.G. Mallozzi, Ph.D. ANAEROBE SOCIETY MEMBERSHIP MEETING WINE & CHEESE RECEPTION / LEGISLATIVE TERRACE

xviii

xix



Congress Program

Wednesday, July 13

8 REGISTRATION / BREAKFAST / EXHIBITS

SESSION VII: ORAL ABSTRACTS: MICROBES ON THE MUCOSA

Convener: Yiping W. Han, Ph.D.

SVII-1 Electronic Nicotine Delivery Systems and the Oral Microbiome:

An Integrated-Omics Analysis

Sukirth Ganesan

SVII-2 Computational Analysis of Disease-Associated Functional Shifts

in the Periodontal Microbiome

Shareef M. Dabdoub, Ph.D.

SVII-3 Effects of Pregnancy and Smoking on the Subgingival Microbiome

Akshay D. Paropkari, Ph.D.

SVII-4 Galectins in the Distal Reproductive Tract: A Novel Mechanism

of Anaerobe Synergisms

Raina N. Fichorova, M.D., Ph.D.

845-905 BREAK / EXHIBITS

SESSION VIII: ESCMID-ASA JOINT SYMPOSIUM: FOCUS ON ORAL ANAEROBES

Convener: Elisabeth Nagy, M.D., Ph.D.

SVIII-1 Highlights on Anaerobe Research in ESCMID

Elisabeth Nagy, M.D., Ph.D.

SVIII-2 Relationship between Methanogenic Archaea and Subgingival

Microbial Complexes in Human Periodontitis

Georg Conrads, Ph.D.

SVIII-3 Disruption of Immune Homeostasis in Pathogen Induced Vascular

Inflammation

Caroline Attardo Genco, Ph.D.

SVIII-4 Diabetes and the Oral Microbiome

Purnima Kumar, D.D.S, Ph.D.

1045-1100 BREAK / EXHIBITS

SESSION IX: WHAT'S IN A NAME: TAXONOMY AND THE ANAEROBES

Convener: Kerin Tyrrell

SIX-1 The Taxonomy of the Genus *Clostridium*: Current Status and Future

Perspectives for the Clinical Community

Paul Lawson, Ph.D.

SIX-2 Reconciling Phenotype vs. Genotype in the Taxonomic Classification

of Anaerobes

Kathy Bernard, M.Sc.

Congress Program



1145-1300 LUNCH / EXHIBITS / AUTHORSHIP WORKSHOP

1300-1400 POSTER SESSION II / EXHIBITS

SESSION X: BACTERIAL REPLACEMENT THERAPY INCLUDING FECAL MICROBIOME TRANSPLANTS

Convener: Emma Allen-Vercoe, Ph.D.

SX-1 Microbiota and Mental Health: Hype or Hope? *Jane A. Foster, Ph.D.*

SX-2 Fecal Microbiota Transplant for Ulcerative Colitis *Michael Surette, Ph.D.*

SX-3 Fecal Microbiota Transplant in Clinical Practice *Colleen Kelly, M.D.*

SX-4 Feasibility of a Room Temperature Stable, Orally Delivered Microbiota Capsule for the Prevention of Recurrent *Clostridium difficile* Infection

Shiva Garg, Ph.D.

SX-5 Host-Specific Fecal Microbiota Transplantation Is More Effective in Treating Recurrent *Clostridium difficile* Infection in a Murine Model *Anna M. Seekatz, Ph.D.*

SX-6 SYN-004: A Pioneering Therapeutic to Protect the Microbiome from Antibiotic-Mediated Damage

Sheila Connelly, Ph.D.

1600-1610 BREAK / EXHIBITS

SESSION XI: INTERACTIONS OF GENITAL TRACT ANAEROBES & RELATIONSHIP TO HUMAN DISEASE

Convener: Jeanne Marrazzo, MD.

SXI-1 Comparative Genomics of Vaginal Anaerobes and Linkage to Metabolites *in vivo* and *in vitro*Sharon Hillier, Ph.D.

SXI-2 Comparative Genomics of Anaerobes and Linkage to Metabolites *Sujatha Srinivasan, Ph.D.*

SXI-3 Cultivation of Vaginal Anaerobes in Epithelial Cells and Associated Models

Richard B. Pyles, Ph.D.

SXI-4 Bacterial Species Colonizing the Vagina of Healthy Women Are Not Associated with Race

May A. Beamer

1830 BANQUET RECEPTION / NASHVILLE CITY CLUB

CONGRESS BANQUET & AWARDS

FINEGOLD AWARD • YOUNG INVESTIGATORS AWARDS LIFETIME ACHIEVEMENT AWARD: Bennett Lorber, Philadelphia, PA 00-1600

1610-1740

1915



Congress Program

Thursday, July 14

8 REGISTRATION / BREAKFAST / EXHIBITS

SESSION XII: ORAL ABSTRACTS: CLOSTRIDIUM DIFFICILE I

Convener: Karen Carroll, M.D.

SXII-1 Re-Examining the Germination Phenotypes of Several *Clostridium difficile* Strains

Joseph A. Sorg, Ph.D.

SXII-2 Clathrin-Independent Endocytosis of Clostridium difficile Toxin A Ramyavardhanee Chandrasekaran

SXII-3 CdtR: [Only?] The Regulator of Binary Toxin in *Clostridium difficile?* Sarah Kuehne, Ph.D.

SXII-4 Leptin Signaling Alters Neutrophil Homeostasis during *Clostridium difficile* Infection

Rajat Madan, M.D.

SXII-5 Intraspecific Competition and Adaptive Immune Responses in Protection Against Murine *Clostridium difficile* Infection *Vincent Young, M.D.*

830-845 BREAK / EXHIBITS

SESSION XIII: EVOLVING MANAGEMENT OF CLOSTRIDIUM DIFFICILE INFECTION

Conveners: Dale Gerding, M.D., Stuart Johnson, M.D.

SXIII-1 Antibiotics: New and in Clinical Research Trials Stuart Johnson, M.D.

SXIII-2 Vaccines to Prevent Clostridium difficle Infections
Chandrabali Ghose-Paul, Ph.D.

SXIII-3 Biotherapeutics and Immunologics for CDI Prevention *Dale Gerding, M.D.*

SXIII-4 Safety and Efficay of RBX2660 for Recurrent Clostridium difficile Infection: Results of Phase 2 Punch CD Studies

Erik R. Dubberke, M.D.

1015-1025 BREAK / EXHIBITS

SESSION XIV: CLINICAL EXPERIENCE OF CLOSTRIDIUM DIFFICILE IN EUROPE

Convener: Carl Erik Nord, M.D., Ph.D.

SXIV-1 Clinical Experience of *Clostridium difficile* in Europe: Introduction; Historical Perspectives

Carl Erik Nord, M.D., Ph.D.

SXIV-2 Diagnosis and Epidemiology of CDI in Europe *Ed Kuijper, M.D., Ph.D.*

SXIV-3 Treatment of Primary and Recurrent CDI in Europe *Mark Wilcox, M.D.*

Congress Program



1200-1320 LUNCH / EXHIBITS

1320-1420 POSTER SESSION III / EXHIBITS

SESSION XV: AN UPDATE ON CLOSTRIDIUM DIFFICILE PATHOGENESIS

Conveners: David M. Aronoff, M.D., Dena Lyras, Ph.D.

SXV-1 Quorum Sensing in Clostridium difficile Charles Darkoh, Ph.D.

SXV-2 Adherence Mechanisms of *Clostridium difficile* to Gut Epithelial Cells *Gayatri Vedantam, Ph.D.*

SXV-3 Unique Contributions of TcdA and TcdB to *Clostridium difficile* Disease

Dena Lyras, Ph.D.

SXV-4 The Impact of Nutrition on *Clostridium difficile* Infection *Joseph P. Zackular, Ph.D.*

1600-1615 BREAK / EXHIBITS

SESSION XVI: ORAL ABSTRACTS: CLOSTRIDIUM DIFFICILE II

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

SXVI-1 Acute Gastroenteritis in Children Using Multiplex Nucleic Acid-Based Testing and the Role of Clostridium difficile Maribeth Ruth Nicholson, M.D.

SXVI-2 Oral Immunization with Non-Toxic *C. difficile* Strains Expressing Chimeric Fragments of TcdA and TcdB Elicit Protective Immunity Against *C. difficile* Infection in Both Mice and Hamsters

Yuanguo Wang

SXVI-3 Clostridium difficile Infection in South-East Asia Thomas V. Riley, Ph.D.

SXVI-4 Disease Progression and Resolution in Rodent Models of *Clostridium difficile* Infection: Impact of Antitoxin Antibodies *Alex G. Therien, Ph.D.*

SXVI-5 Dissecting the Assembly Mechanism and Functional Role of the Outermost Exosporium Layer of *Clostridium difficile* Spores *Daniel Paredes-Sabja, Ph.D.*

CONGRESS CONCLUDES



xxii

xxiii

1615-173



Oral Abstract Contents



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

	Contents	Abstracts
Resistant Infections in Era of Antimicrobial Stewardship	3	4-7
Keynote Address	9	10
Non-difficile Clostridia	11	12-16
Approaches for Personalized Therapuetics	17	18-20
Literature Review in Anaerobic Sciences	21	22-23
Oral Abstract Potpourri	25	26-28
Microbes on the Mucosa	29	30-33
Oral Anaerobes	35	36-39
Taxonomy and the Anaerobes	41	42-43
Bacterial Replacement Therapy	45	46-51
Genital Tract Anaerobes	53	54-57
Oral Abstracts: Clostridium difficile I	59	60-64
Management of C. difficile Infection	65	66-69
Clostridium difficile in Europe	71	72-74
Clostridium difficile Pathogenesis	75	76-79
Oral Abstracts: Clostridium difficile II	81	82-86

xxiv 1



Poster Abstract Contents

	Contents	Abstracts
Poster Presentations: Session I		
Clinical Abstracts	87	89-104
Non-difficile Clostridia	105	106-114
Gut Microbiome	115	116-124
Student Poster Presentations	125	126-134
Poster Presentations: Session II		
Anaerobes in the Mouth	135	136-145
Anaerobes in the Genital Tract	147	148-151
Anaerobe Microbiology	153	154-162
Probiotics	163	164-171
Clostridium difficile: Pathogenesis	173	175-187
Poster Presentations: Session III		
Clostridium difficile: Epidemiology	189	191-203
Clostridium difficile: Microbiology	205	207-220
Clostridium difficile: Management	221	223-236

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 PIII—Poster Presentation/Session III

SP—Student Presentation

*Indicates Presenting Author

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

Tuesday, July 12, 2016

Resistant Infections

925-945 SESSION I: TREATING MULTI-DRUG RESISTANT INFECTIONS IN THE ERA OF ANTIMICROBIAL STEWARDSHIP

SI-1	The Clinical Presentation of Fusobacterium necrophorum	
	Pharyngitis	4
	Centor, R.M.*	
SI-2	The New Agents for Complicated Intra-Abdominal Infections: What are the Trials Saying? Solomkin, J.S.*	5
SI-3	Parallels between <i>C. difficile</i> Colitis and Autism <i>Finegold, S.M.</i> *.	6
SI-4	The Non-existent Drug Pipeline for Anaerobic Infections Goldstein, E.I.C.*	7

SI-1

THE CLINICAL PRESENTATION OF FUSOBACTERIUM NECROPHORUM PHARYNGITIS

Centor, R.M.* University of Alabama, Huntsville, AL USA

Research has suggested that *Fusobacterium necrophorum*, an obligate gramnegative anaerobic bacteria, caused endemic pharyngitis in adolescents and young adults. Studies, using PCR or anaerobic cultures, found that approximately 10% of endemic pharyngitis in the 15-30 age group tested positively for *F. necrophorum*.

Interest in *F. necrophorum* occurred because over 80% of Lemierre Syndrome patients have positive blood cultures for this bacterium. As this devastating syndrome has increased in frequency over the past 2-3 decades, several researchers have opined that we should work to prevent the syndrome through treating this bacterial pharyngitis. The Lemierre Syndrome starts with a sore throat and fever. Unlike most pharyngotonsillitis, patients have sore throats that continue to worsen. Within days, they develop rigors and then a unilateral suppurative internal jugular thrombophlebitis. These clots shower emboli, often causing multiple pulmonary septic abscesses, but sometimes also infecting the brain, the liver, or the joints.

More recently, peritonsillar abscess studies have shown that *F. necrophorum* is the most common bacteria found (especially in the 15-30 age group).

While previous studies have estimated the prevalence of *F. necrophorum* in 15-30 year old patients presenting with a sore throat, several studies in 2015 sought to describe its clinical presentation. Kjaerulff and colleagues from Denmark found that 24% of sore throats in patients' aged 15-29 grew *F. necrophorum*, while 9% of controls in that age group grew the organism. They did not find any clinical predictors of *F. necrophorum* in their small study. Hedin and colleagues from Sweden in a larger study found that the average Centor score (1 point each for tonsillar exudates, swollen tender anterior cervical adenopathy, fever history and lack of cough) was higher in both Group A streptococcal (GAS) pharyngitis and *F. necrophorum* pharyngitis.

Finally, our recent study used PCR techniques to identify GAS, group C/G strep and *F. necrophorum*. We found that all 3 bacterial species occur more often with higher Centor scores. These findings, along with our previous report of 6 patients with bacteremic *F. necrophorum* pharyngitis, suggest strongly that the Centor score predicts (albeit imperfectly) bacterial pharyngitis—and not just GAS pharyngitis. We endorse strategies of treating high probability bacterial pharyngitis to decrease non-suppurative (acute rheumatic fever) and suppurative complications (peritonsillar abscess and the Lemierre syndrome).

THE NEW AGENTS FOR COMPLICATED INTRA-ABDOMINAL INFECTIONS: WHAT ARE THE TRIALS SAYING?

Solomkin, J.S.*

University of Cincinnati College of Medicine, Cincinnati, OH USA

Despite concerns that antibiotic development has faltered, new agents continue to appear. Three phase 3 trials in complicated intra-abdominal infections (cIAI) have been completed, and two have resulted in the regulatory approval of ceftolozane/tazobactam (TOLT) and ceftazidime/ avibactam (CAZA). Eravacycline is awaiting resolution of a failed UTI study. The target of recent drug development has been the rapidly emergent extended spectrum beta-lactamase (ESBL) containing gram-negatives and carbapenemase producing Enterobacteriaceae (CRE) and specific pathogens (Pseudomonas aeruginosa and Acinetobacter baumannii complex. Other new antibiotics include carbapenems with novel Beta-lactamase inhibitors and an aminoglycoside. TOLT and CAZA have only modest anaerobic activity, and therefore metronidazole (MTZ) was added to avoid potential confounding. Microbiology assessments in these trials have also changed towards capturing facultative and aerobic gram-negatives and relying on local laboratories for anaerobic identification. These more recent studies are, therefore, underweighted for anaerobic pathogens vs. previous studies. Further, these studies generally did not capture sufficient numbers of ESBL or CRE containing microorganisms to evaluate effectiveness against these pathogens. The reported trials demonstrate particular problems with both statistical outcomes expressed as odds ratios and confidence intervals.

A further concern in these trials is that resistant pathogens to the provided therapy, particularly *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex, were eradicated with considerable efficiency.

Conclusions: It is unclear where newer agents will fit in the management of cIAI. It is apparent that the antimicrobial requirements for polymicrobial infections need to be revisited. It is not apparent that this indication provides a clear basis for regulatory approval of new antimicrobials.

SI-3

PARALLELS BETWEEN C. DIFFICILE COLITIS AND AUTISM

Finegold, S.M.*

VA Medical Center West Los Angeles, Los Angeles, CA USA

Some two-thirds of new cases of autism are of the regressive variety and follow exposure to antimicrobial agents. The organisms from the bowel flora associated with autism of this type include *Clostridium bolteae*, other clostridial species, *Desulfovibrio* species, and *Sutterella* species. In some cases, toxins are involved; in others, it is metabolites. The classic presentation is an ear, upper respiratory, or other infection in a child 12-18 months of age treated with antimicrobials (penicillins, cephalosporins, sulfonamides, and others), followed by regression of speech, social behavior, toilet training, etc. and, in many cases, gastrointestinal issues (primarily constipation) characteristic of autism. The above microorganisms associated with this type of autism are resistant to the antimicrobials administered, just as *Clostridium difficile* is.

One small study with open label oral vancomycin led to clearance or improvement in the above manifestations, while the treatment was continued and relapse after it was stopped. This was a pilot study to demonstrate that an appropriate antimicrobial could reverse the clinical picture and that therefore the recovery of the indicated organisms was indicative of their role in the process. It is my feeling that antimicrobials should not be used in therapy of autism, however. There are other options to be discussed.

The widespread use of antimicrobials in children and the significant incidence of resistance to these drugs may be part of the reason for the remarkable increase in incidence of autism in recent years and the increased incidence of multiple cases of autism in families. Thus, this type of autism may properly be considered a side effect of antimicrobial therapy just as *C. difficile* colitis is.

THE NON-EXISTENT DRUG PIPELINE FOR ANAEROBIC INFECTIONS

Goldstein, E.J.C.*

R.M. Alden Research Laboratory, Santa Monica, CA USA

While anaerobic infections continue to be a clinical problem worldwide and the emergence of resistance of veterinary and human clinical isolates continues to expand, including more reports of *Bacteroides fragilis* group resistance, there is a paucity of new drugs for the therapy of anaerobic infections. Most new anaerobic drugs are now targeting *C. difficile* infections. This presentation will review the *in vitro* activity and available clinical data of the following and other agents in development.

Most new compounds being tested are combinations of old (e.g., ceftazidime, ceftaroline, imipenem, meropenem) or new (e.g. ceftolozane) beta-lactam agents in combination with an old or new beta-lactamase inhibitors (avibactam, relebactam, RPX7009, RG6080). Most are targeted towards their activity against new aerobic gram-negative MDROs (multidrug resistant organisms). Ceftolozane—tazobactam and eftazidime-avibactam are marketed for cIAI. Other compounds are undergoing clinical evaluations in the therapy of intra-abdominal infections. Several older fluoroquinolones as pazufloxacin, and delafloxacin have appeared in Abstracts at ICAAC 2015.

Eravacycline (TP-434), a novel fluorocycline, has been evaluated *in vitro* against anaerobic bacteria and has successfully completed a comparative phase III study in community intra-abdominal infections against ertapenem.

Other classes of compounds as Antimicrobial peptides, Fab 1 inhibitors and siderophore antimicrobials have no published data on their anaerobic activity.



Tuesday, July 12, 2016

Keynote Address

1000 SESSION II: KEYNOTE ADDRESS

SII-1 Never Let a Crisis Go to Waste: Progress in Prevention of Clostridium difficile Transmission Donskey, C.J.*

10

8

Tuesday, July 12, 2016

1100

Non-difficile Clostridia

NEVER LET A CRISIS GO TO WASTE: PROGRESS IN PREVENTION OF CLOSTRIDIUM DIFFICILE TRANSMISSION

Donskey, C.J.*1,2 ¹Louis Stokes VA Hospital, Cleveland, OH USA ²Case Western Reserve University, Cleveland, OH USA

The basic measures used to prevent transmission of *Clostridium difficile* include placement of infected patients in contact precautions until diarrhea resolves and disinfection of surfaces and equipment with sporicidal products such as sodium hypochlorite. Unfortunately, these basic measures have often proven ineffective during the past decade, as infection rates have risen dramatically in the setting of large outbreaks due to 027/BI/NAP1 C. difficile strains. This presentation will review recent evidence challenging traditional models of transmission and consider new approaches to reduce C. difficile transmission. These approaches include novel strategies to reduce the burden of spores in the environment and on skin and to prevent transmission by asymptomatic carriers. The potential impact of diagnostic testing approaches and antimicrobial stewardship on transmission will also be discussed.

10

	CLOSTRIDIA AND INTRA-ABDOMINAL INFECTIONS	
SIII-1	Diet Fiber Contributes to Peritonitis by Activating Inflammasome NLRP3 and Inducing IL-1 β Secretion	12
	Castelpoggi, J.P.; de Almeida, B.J.; Dias, M.; Ramos Jr., E.S.; Ferreira, E.O.; Domingues, R.M.; Vieira, L.Q.; Mariño, E.; Mackay, C.R.; Coutinho-Silva, R.; Zamboni, D.; Scharfstein, J.; Bellio, M.; Lobo, L.A.;* Oliveira, A.C.	
SIII-2	Detection of Toxigenic <i>Clostridium perfringens</i> and <i>Clostridium botulinum</i> from Food Commodities in Lagos State, Nigeria	13
	Chukwu, E.E.; Nwaokorie, F.O.;* Coker, A.O.; Avila-Campos, M.J.; Solis, L.; Llanco, L.A.; Ogunsola, F.T.	
SIII-3	Purification and Properties of a Novel Botulinum Neurotoxin FA "H"	14
	Johnson, E.A.;* Tepp, W.H.; Pellet, S.; Bradshaw, M.; Nawrocki, E.M.; Lin, G-L.; Kalb, S.; Pier, C.L.; Barr, J.R.; Maslanka, S.	
SIII-4	Pathology of Bacillary Hemoglobinuria Produced by Clostridium haemolyticum	15
	Navarro, M.;* Dutra, F.; Romero, A.; Briano, C.; Persiani, M.; Uzal, F.A.	
SIII-5	The Comparative Efficacy of Antibiotics against Experimental <i>Clostridium septicum</i> Infection	16
	Aldape, M.J.; * Bayer, C.R.; Rice, S.N.; Bryant, A.E.; Stevens, D.L.	

11

SESSION III: ORAL ABSTRACTS: NON-DIFFICILE

SIII-1 SIII-2

DIET FIBER CONTRIBUTES TO PERITONITIS BY ACTIVATING INFLAMMASOME NLRP3 AND INDUCING IL-1\$ SECRETION

Castelpoggi, J.P.;¹ de Almeida, B.J.;¹ Dias, M.;² Ramos Jr., E.S.;¹ Ferreira, E.O.;² Domingues, R.M.;² Vieira, L.Q.;³ Mariño, E.;⁴ Mackay, C.R.;⁴ Coutinho-Silva, R.;¹ Zamboni, D.;⁵ Scharfstein, J.;¹ Bellio, M.;² Lobo, L.A.;*² Oliveira, A.C.¹

¹Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brazil ²Instituto de Microbiologia Paulo de Góes, UFRJ, Rio de Janeiro, Brazil ³Faculdade de Medicina de Ribeirão Preto, USP, São Paulo, Brazil ⁴Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil

Peritonitis is an inflammation in the peritoneal cavity that may be caused by abdominal trauma or any perforation of the gut epithelium followed by spillage of intestinal content. Bacteria from microbiota and diet products spread into the peritoneal cavity and lead to inflammation. Applying a widely used monomicrobial model of peritonitis induced by B. fragilis and sterile cecal content (SCC), we show that IL-1β is essential for this inflammatory response. SCC alone induces IL-1β production by dendritic cells (DC) and macrophages, which was confirmed in *in vivo* assays, whereas *B. fragilis* induces pro-IL-1β expression, but does not activate the inflamassome pathway. The IL-1β production induced by SCC is totally dependent on NLRP3, ASC and caspase-1, and amplified by autocrine effect through IL-1R. Consistently, NLRP3, ASC, caspase-1 and IL-1R deficient mice are protected from decrease on body weight and exhibit reduced peritonitis score after challenge with SCC plus B. fragilis. Mechanistically, the IL-1ß secretion induced by SCC is abolished by KCl, cytochalasin D, cathepsin inhibitor and partially inhibited in P2X₂-deficient cells, as observed for Alum, suggesting the essential role of non-digestible particulate matter present in the diet on inflammasome activation. Accordingly, mice fed a fiber-restricted diet have reduced IL-1β production whereas SCC from high fiber diet fed mice induces higher IL-1β production in a NLRP3dependent manner. Finally, our results show that purified dietary fiber, Guar Gum, induces caspase-1 activation and IL-1β production in LPS-primed macrophages and dendritic cells in an NLRP3-dependent way. Our results show that the development of peritonitis is dependent on inflammasome/ IL-1β pathway activation by diet products, in special non-digestible fiber, which works as a danger signal outside gut.

DETECTION OF TOXIGENIC CLOSTRIDIUM PERFRINGENS AND CLOSTRIDIUM BOTULINUM FROM FOOD COMMODITIES IN LAGOS STATE, NIGERIA

Chukwu, E.E., Nwaokorie, F.O., Coker, A.O., Avila-Campos, M.J., Solis, L., Llanco, L.A., Ogunsola, F.T.

¹Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria

²Department of Medical Laboratory Sciences, College of Medicine, University of Lagos, Nigeria

³Anaerobe Laboratory, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo-USP, São Paulo, SP, Brazil

⁴Department of Medical Microbiology and Parasitology, Babcock University Teaching Hospital, Ilishan, Remo, Ogun State, Nigeria

Food-borne and waterborne diseases contribute to huge burden of sickness and deaths globally. In the last decade, these diseases are frequently reported in Africa. In line with this, food safety is becoming a significant and growing public health problem in Nigeria. Most studies are focusing on aerobic microbial pathogens associated with diarrhoeal diseases. Toxin producing Clostridium species have been associated with severe food-borne diseases. However, data on food commodities posing as risk factors, Clostridium species implicated, as well as specific toxins associated with food-borne diseases is lacking in Nigeria. This study was carried out to isolate, characterize and detect toxin genes in Clostridium species isolated from food commodities in Lagos, Nigeria. A total of 220 meat containing food commodities, honey, vegetables, and canned foods were collected from three local governments in Lagos State, which was mapped to reflect the high, middle, and low economic levels population. Samples were pre-enriched in Robertson cooked meat medium overnight before inoculating into Clostridium perfringens agar base (CPA), Tryptose Sulfite Cycloserine Agar (TSC), Reinforced Clostridial Agar media and Fastidious anaerobic agar respectively. Isolates obtained were identified based on cultural, morphological, and biochemical characteristics. Toxinotyping was done using multiplex-PCR with primers specific for alpha, beta, epsilon, and iota-toxin genes, enterotoxigenic cpe gene and neurotoxigenic BoNt gene. Of the 220 food sampled, 50 (22.7%) Clostridial species were isolated of which 29 (58%) were identified as C. perfringens. Toxinotyping of the 29 strains showed that 28 (96.6%) were toxin producing *C. perfringens* type A, while one (3.4%) was *C. perfringens* type D. Two (4%) C. botulinum species were isolated and identified by 16S rRNA sequencing, both harbouring BoNt/A gene. Majority of the isolates were obtained from meat products, vegetables, and milk products. Our talk will elaborate on the various toxin genes detected, the different classes of toxin genes harboured by these species and the corresponding food products from which the species were isolated. Consuming unsafe foods poses a significant public health threat in the African Region. Our findings show that toxinogenic clostridia contaminated food products are sold in Lagos markets. The presence of Clostridium species in variety of food products indicate that these potentially hazardous products may be posing a serious public health risk to consumers. It is important to investigate possible clinical cases of Clostridium food borne diseases in the study area. It is also necessary to educate the population on proper food processing in order to facilitate a better hygiene and ensure food safety.

Acknowledgements: This work was supported by the Sigma Delta Epsilon/Graduate Women in Science (SDE/GWIS) Nelly Mondy Fellowship 2014.

PURIFICATION AND PROPERTIES OF A NOVEL BOTULINUM NEUROTOXIN FA "H"

Johnson, E.A.;*1 Tepp, W.H.;¹ Pellet, S.;¹ Bradshaw, M.;¹ Nawrocki, E.M.;¹ Lin, G-L.;¹ Kalb, S.;¹ Pier, C.L.;¹ Barr, J.R.;² Maslanka, S.²

¹Department of Bacteriology, University of Wisconsin, Madison, WI USA ²Centers for Disease Control, Atlanta, GA USA

Botulinum neurotoxins (BoNTs) produced by *Clostridium botulinum* comprise an increasingly diverse group of toxins including variants within a serotype, and hybrid and chimeric toxins between serotypes. In 2014 a strain of Clostridium botulinum (IBCA10-7060; CDC69016) was reported from a case of infant botulism that produced two botulinum neurotoxins (BoNT/B2 and BoNT/FA). Initially, BoNT/FA was reported to constitute a new serotype (serotype "H"), but detailed mouse neutralization studies and genetic sequencing indicated that FA was a hybrid BoNT consisting of a BoNT/ A1 receptor binding domain, a BoNT/F5 catalytic domain, and a complex translocation domain. In this study, we used a Clostron mutant with the BoNT/B2 gene inactivated to singularly produce BoNT/FA, enabling the purification and characterization of the novel chimeric toxin. BoNT/FA was produced in Toxin Production Medium (TPM) by the BoNT/B2tox mutant strain generated by insertional mutagenesis of the wild type CDC69016. The toxin complex was isolated by acid precipitation. BoNT/FA was isolated to greater than 95% purity by RNase treatment, trypsinization, and ion exchange chromatography. Specific toxicity was determined by mouse bioassay and activity in neuronal cell cultures. The specific activity in mice was in the range of other serotypes of BoNTs. BoNT/FA showed an unusual pattern of lethality in mice. BoNT/FA had a high toxicity in human neuronal cells compared to most other serotypes. Having the purified BoNT/FA will enable detailed studies of its mechanism of action and its immunological properties.

14

PATHOLOGY OF BACILLARY HEMOGLOBINURIA PRODUCED BY CLOSTRIDIUM HAEMOLYTICUM

Navarro, M.;*1 Dutra, F.;² Romero, A.;² Briano, C.;² Persiani, M.;¹ Uzal, F.A.¹ ¹California Animal Health and Food Safety Laboratory System, San Bernardino Branch, University of California, Davis, CA USA ²DILAVE Miguel Rubino, Laboratorio Regional Este, Treinta y Tres, Uruguay

Bacillary hemoglobinuria (BH) is an infectious disease that occurs mostly in cattle, caused by *Clostridium haemolyticum*, an anaerobic, sporulated rod. BH is usually fatal and clinically characterized by jaundice, hemoglobinuria, and anemia. The migration of immature forms of the trematode Fasciola hepatica through the liver, producing necrosis and associated anaerobiosis, is believed to be the main predisposing factor for the germination of *C*. haemolyticum spores and the production of the highly necrotic and hemolytic beta toxin, the main virulence factor of this microorganism. We evaluated 20 cases of bovine BH to characterize the pathology of the disease. All carcasses presented jaundice. In all cases, a large, frequently single focus of necrosis was observed mostly on the right or left hepatic lobes; this lesion was pale and surrounded by a red to purple halo. The urinary bladder was filled with dark red urine and the kidneys were diffusely dark brown to black. Microscopically, fibrin thrombi were visualized in branches of portal and hepatic veins within the liver. C. haemolyticum was detected by immunohistochemistry in the hepatic lesions of all animals and by PCR in the liver of two cases that were subjected to this test. Lesions attributable to F. hepatica were not observed in any case. Hemoglobinuric nephrosis and necrotizing splenitis were observed in all cases. Transmission electron microscopy revealed clumps of rods, many of them with subterminal spores, in areas of hepatic necrosis. The predisposing factor was not determined in this series of cases and further research is needed to determine it.

15

THE COMPARATIVE EFFICACY OF ANTIBIOTICS AGAINST EXPERIMENTAL CLOSTRIDIUM SEPTICUM INFECTION

Aldape, M.J.;* Bayer, C.R.; Rice, S.N.; Bryant, A.E.; Stevens, D.L. Veterans Affairs Medical Center, Boise, ID USA

Clostridium septicum is a highly pathogenic microbe that causes gas gangrene in humans and is the principal cause of spontaneous gas gangrene in patients with gastrointestinal maladies, including adenocarcinoma of the colon. Despite modern approaches to manage C. septicum infections, morbidity and mortality remain high (>50%). At present, no objective in vivo data exists supporting the current antibiotic treatment recommendations for C. septicum infection. Utilizing our established murine model of clostridial myonecrosis, we investigated the efficacy of standard antibiotics for anaerobic Gram positive soft-tissue infections (penicillin, clindamycin, tetracycline, vancomycin) in treating C. septicum gas gangrene. Following intramuscular challenge with 1x106 colony forming units of C. septicum, antibiotics were administered by intraperitoneal injection every four hours for a total of four doses. At 30 hours, all animals in all treatment groups survived the *C. septicum* challenge, compared to no survivors in the untreated controls (100% mortality by 10 hours). By 60 hours, mice treated with vancomycin exhibited 40% mortality, with no mortality observed in any other antibiotic treatment group. Additionally, microbroth dilution MIC analysis for three strains of C. septicum collectively demonstrated high susceptibility to penicillin, clindamycin and tetracycline, but considerably lower susceptibility to vancomycin. These studies corroborate that penicillin, clindamycin, and tetracycline may be suitable alternatives for the treatment of C. septicum infection in humans.

<i></i>	, , , , , , , , , , , , , , , , , , , ,	
1415	SESSION IV: DEFINING THE FUNCTION OF THE GUT USING OMIC APPROACHES FOR RATIONAL DESIGN OF PERSONALIZED THERAPEUTICS	
SIV-1	Rational Design of Microbiota-Mediated Secondary Bile Acids in the Gut to Restore Colonization Resistance against <i>C. difficile Theriot</i> , <i>C.M.;* Thanissery</i> , <i>R.S.; Winston</i> , <i>J.A.</i>	18
SIV-2	Nutrimetabonomics to Understand Host-Pathogen Response to Antibiotic Treatment LeRoy, C.I.; Woodward, M.J.; La Ragione, R.M.; Claus, S.P.*	19
SIV-3	Methanogens in the Gut and Their Interactions with Beneficial Butyrate Producers	20

Sieber, J.R.;* Schmidt, T.M.

Approaches for Personalized Therpeutics

Tuesday, July 12, 2016

16 17

RATIONAL DESIGN OF MICROBIOTA-MEDIATED SECONDARY BILE ACIDS IN THE GUT TO RESTORE COLONIZATION RESISTANCE AGAINST CLOSTRIDIUM DIFFICILE

Theriot, C.M.;* Thanissery, R.S.; Winston, J.A. Department of Population Health and Pathobiology, College of Veterinary Medicine North Carolina State University Raleigh, NC USA

Members of the gut microbiota are responsible for converting primary bile acids into secondary bile acids, which are known to inhibit C. difficile growth *in vitro*. Based on this our *hypothesis* is the production of secondary bile acids by the gut microbiota contributes to colonization resistance against C. difficile. Using targeted bile acid metabolomics, we sought to define the physiologically relevant concentrations of primary and secondary bile acids present in the murine small and large intestinal tract and how this affected *C. difficile* dynamics. We treated mice with a variety of antibiotics to create distinct microbial and metabolic (bile acids) environments, and directly tested their ability to support or inhibit C. difficile spore germination and outgrowth ex vivo. Susceptibility to C. difficile in the large intestine was observed only after treatment with specific antibiotics (cefoperazone, clindamycin and vancomycin) and was accompanied by a significant loss of secondary bile acids (DCA, LCA, UDCA, HDCA, ωMCA and iLCA). These changes were associated with a loss of gut bacteria from the Lachnospiraceae and Ruminococcaceae families. Additionally, physiological concentrations of secondary bile acids present during C. difficile resistance were able to inhibit spore germination and outgrowth of many clinically relevant *C. difficile* strains in vitro. Interestingly, we observed that C. difficile spore germination and outgrowth was supported constantly in the murine small intestine regardless of antibiotic perturbation, suggesting that targeting growth of C. difficile will prove important for future therapeutics. Future directions focusing on biochemical characterization of bile acid-altering gut microbes will give us the ability to rationally design the bile acid composition in the gut. Understanding how the gut microbiota regulates bile acids throughout the intestine will aid the development of future therapies for *C. difficile* infection and other metabolically relevant disorders such as obesity and diabetes.

NUTRIMETABONOMICS TO UNDERSTAND HOST-PATHOGEN RESPONSE TO ANTIBIOTIC TREATMENT

LeRoy, C.I.;¹ Woodward, M.J.;¹ La Ragione, R.M.;² Claus, S.P.*¹
¹Department of Food & Nutritional Sciences, The University of Reading, Whiteknights Campus, Reading UK
²Faculty of Health and Medical Sciences, School of Veterinary Medicine, The University of Surrey, Guilford, Surrey UK

The gut microbiota is now recognized as a fundamental partner for maintaining the host's health status. Normally, the host-microbiota symbiosis results in a healthy metabolic phenotype. However, as the environment changes, our metabolism adapts to maintain homeostasis within an optimal metabolic space, and so do our microbiota. So how does this interplay result in an optimal metabolic state? And how can this be measured? Nutrimetabonomics is a useful tool to assess the metabolic state of the host in response to environmental perturbations. The studies presented here will illustrate how nutrimetabonomics can be used to gain an understanding of the metabolic disruptions triggered by *Brachyspira pilosicoli*infection in poultry. We will discuss how a better knowledge of the host metabolic response to a pathogen and antibiotic treatment, can aid in the design of new therapeutic alternatives to antibiotics.

Tuesday, July 12, 2016

Anaerobic Sciences

METHANOGENS IN THE GUT AND THEIR INTERACTIONS WITH BENEFICIAL BUTYRATE PRODUCERS

Sieber, J.R.;*1 Schmidt, T.M.²
¹University of Minnesota, Duluth, MN USA
²University of Michigan, Ann Arbor, MI USA

Metabolic interactions between hosts and their gut microbiomes have evolved to be fundamental to host health. Interruptions to these networks, through changes to the community structure or function, increasingly are recognized as contributing to many human diseases. Microbial production of short chain fatty acids (SCFAs) via fermentation within the large intestine is important to the host, as SCFAs can serve as an energy source for epithelial cells, act as signaling molecules and regulates carbohydrate resistance and fat storage. Butyric acid—the primary four-carbon SCFA produced in the gut—has anti-inflammatory properties, prevents colon carcinogenesis and induces satiety by stimulating the production of GLP-1. The large intestine of nearly all humans is colonized primarily by a single methanogenic species, Methanobrevibacter smithii. This methanogenic archaeon has a streamlined metabolism relying primarily on the consumption of hydrogen to reduce carbon dioxide to methane. In microcosms derived from mouse and human fecal microbiomes, we found that butyrate production is inhibited when the headspace concentration of H₂ is increased. We also found that the addition of the hydrogen-consuming methanogen, Methanobrevibacter smithii, to the microcosms increased the production of SCFAs, including butyrate. These results are consistent with H₂ inhibiting the reoxidation of NADH by hydrogenases, including those linked to the production of butyrate. By consuming hydrogen, hydrogenotrophic methanogens can be important for maintaining large intestine health.

1545	SESSION V: LITERATURE REVIEW IN ANAEROBIC SCIENCES	
SV-1	An Update on <i>Fusobacterium</i> Disease Pathogenesis <i>Sears, C.L.</i> *	22
SV-2	Bacteroides: Beyond the Microbiome	23
	Tillotson, G.S.*	

SV-1 SV-2

AN UPDATE ON FUSOBACTERIUM DISEASE PATHOGENESIS

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

While Fusobacterium spp. have long been known to be invasive anaerobes of importance in head and neck, as well as abdominal infections, a member of this genus, Fusobacterium nucleatum, gained prominence in recent years through its association by 16S rRNA sequencing analyses with colorectal cancer. This session will provide an overview of recent Fusobacterium spp. literature. Specifically, recent human clinical observations will be presented and the audience will be provided a perspective on the data associating the Fusobacterium genus and, in particular, F. nucleatum, with human carcinogenesis.

BACTEROIDES: BEYOND THE MICROBIOME

Tillotson, G.S.* Cempra Pharmaceuticals Inc., Chapel Hill, NC USA

As with many other bacteria the "real" role of the Gram negative genus Bacteroides has become clearer, however there is increasing new information of pathogenicity mechanisms and novel aspects of the genus members which provide insights into how they maintain a role in the "normal" microbiome and yet also play a key role in infective processes. In this presentation, data on both new microbiome assessments and pathogenicity attributes of Bacteroides species will be discussed.



Tuesday, July 12, 2016

1645	SESSION VI: ORAL ABSTRACTS: POTPOURRI	
SVI-1	Taking the Next Giant Step: Designed Microbiome Therapeutics for <i>Clostridium difficile</i> Infection (CDI)	26
	Cook, D.N.;* Lombardo, M-J.	
SVI-2	'In Silico' Chemotaxonomy: A Tool for Microbial Systematics	27
	Patel, N.B.;* Sankaranarayanan, K.; Lawson, P.A.	
SVI-3	First Genome Sequence of the Opportunistic Pathogen Clostridium septicum	28
	Ajami, N.J.; Holder, M.E.; Roxas, B.P.; Mallozzi, M.J.G.;* Lewis, D.E.; Vedantam, G.; Petrosino, J.F.	

Oral Abstracrs: Potpourri

TAKING THE NEXT GIANT STEP: DESIGNED MICROBIOME THERAPEUTICS FOR CLOSTRIDIUM DIFFICILE INFECTION (CDI)

Cook, D.N.; Lombardo, M-J.* Seres Therapeutics, Inc., Cambridge, MA USA

Patients with *C. difficile* infection are at risk for recurrence because targeted antibiotics do not eradicate dormant spores, nor address the underlying dysbiosis. Thus, CDI places an enormous burden on US healthcare systems due to a vicious cycle of antibiotic re-exposure, clinical relapse and forward transmission to others.

Fecal microbiota transplantation (FMT) has been effective in the prevention of recurrent CDI, providing a proof-of-principle that the human microbiome may be suitable therapeutic target. However, FMT may serve as a transmission vehicle for any emerging infection within the community due to minimal processing.

SER-109 is an ecology of Firmicute spores from healthy screened stool donors. Based on pre-clinical efficacy in rodent CDI models, SER-109 was developed based on the hypothesis that spore-forming organisms would compete metabolically with *C. difficile* for essential nutrients and / or bile acids. Spores are also stable and resistant to stomach acid, facilitate consistency of dosing, and can be harvested from a small number of stool donors. Finally, the manufacturing process of SER-109 includes ethanol inactivation of bacteria, parasites, fungi, and most viruses, which improves its safety profile compared to donor screening alone. In a phase 1B/2 trial, SER-109 was effective in preventing CDI recurrence in subjects with a history of multiple recurrent CDI. In parallel, microbial diversity increased through engraftment of spores and augmentation of microbes that were absent in the study subject at baseline or present in low numbers. Although the safety profile of SER-109 is markedly enhanced compared to whole stool FMT, patient risk may be further mitigated by eliminating human source material altogether. SER-262 is a second-generation microbiome therapeutic, produced by in vitro fermentation of bacterial strains for the prevention of CDI recurrence in subjects with a primary episode. The methodology of microbe selection and representative animal testing data from more than 100 combinations will be presented.

'IN SILICO' CHEMOTAXONOMY: A TOOL FOR MICROBIAL SYSTEMATICS

Patel, N.B.;*1Sankaranarayanan, K.;2 Lawson, P.A.1

1Department of Microbiology & Plant Biology

2Department of Anthropology, University of Oklahoma, Norman, Oklahoma, USA

The characterization of novel microorganisms traditionally requires a polyphasic approach that includes phylogenetic, biochemical and chemotaxonomic investigations. Analytical tests for characterizing chemotaxonomic biomarkers are typically labor intensive and expensive. This, coupled with the lack of curated databases and standardized protocols, makes reproducibility of these tests and comparative analysis of results difficult. Alternatively, genomic information, specifically the identification of genes involved in metabolic pathways associated with the biosynthesis of these taxonomic markers can serve as a powerful tool for classification.

We hypothesized that this "in silico" chemotaxonomy can complement existing laboratory protocols, to aid in the identification of microorganisms. We evaluated this hypothesis using polar lipids as a model, comparing published TLC/Mass spectrometry data with genomic annotations for fifty bacterial strains representing six major phyla. We identified several trends between the in silico predictions and published in vitro characterizations, ranging from complete concordance (across all taxa examined) for the polar lipid Phosphatidylethanolamine, to phylum specific concordance as observed for the polar lipid Diphosphatidylglycerol among Firmicutes.

Collectively, these results highlight several challenges currently facing "in silico" approaches to chemotaxonomy. These include (a) the need for experimental characterization of biosynthesis pathways in a phylogenetically diverse range of organisms, and, (b) standardized reporting for "in vitro" characterizations of taxonomic markers. Addressing these challenges will allow for more refined hypothesis testing, and generation of phylogenetically specific sequence models and curated databases for "in silico" markers, providing reliable chemotaxonomic information that is accessible to the larger scientific community.

FIRST GENOME SEQUENCE OF THE OPPORTUNISTIC PATHOGEN CLOSTRIDIUM SEPTICUM

Ajami, N.J.; Holder, M.E.; Roxas, B.P.; Mallozzi, M.J.G.; Lewis, D.E.; Ajami, N.J.; Holder, M.E.; Roxas, B.P.; Mallozzi, M.J.G.; Vedantam, G.;2,5 Petrosino, J.F.1

¹Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX USA

²University of Arizona, School of Animal & Comparative Biomedical Sciences, Tucson, AZ USA

³The Gut Check Foundation, Tucson, AZ USA

⁴The University of Texas Health Science Center, Department of Internal Medicine, Houston, TX USA

⁵BIO5 Research Institute, Tucson, AZ USA

Clostridium septicum (CS) is a recalcitrant opportunistic pathogen responsible for fatal wound and gastrointestinal tract infections. CS infection of the GI tract is often associated with colorectal cancer and malignancies involving the bowel (especially leukemias), and diagnosis is complicated by an overlap between diagnostic symptoms (tachycardia and pain without identifiable cause) and the side effects of chemotherapy. Currently, a rapid diagnostic platform for CS infection does not exist, and research on this organism has been hampered by a lack of genetic tools and genomic sequence.

Study Purpose and Methods: The goal of this study was to obtain a full, high-quality genome sequence for this devastating pathogen. A novel combination of PacBio and 10kB Nextera Mate Pair library Illumina sequencing platforms were employed. Hybrid assembly was accomplished using the SPAdes and QUIVER algorithms, and the genome annotated using Rapid Annotation using Subsystems technology (RAST).

Results: A 3.4Mb chromosome and a 5kb plasmid harboring multiple antibiotic resistance genes were identified. A significant proportion of the genome shared similarity with other Clostridia; however, two regions were unique to the species. One, (a 50kb DNA segment) bearing the hemolytic aerolysin-like alpha toxin gene (a known virulence determinant), and the other (a 92kb region) encoding a novel multi-domain, putatively secreted 206kDa protein of unknown function. Analysis of the annotated genome revealed a large number of genes dedicated to fermentation (59), cell wall and capsule biosynthesis (99), motility (48), sporulation (76), oxidative stress (31), and virulence (60).

Conclusion: CS harbors multiple sequences likely dedicated to host adaptation during infection. It is anticipated that the public availability of this CS sequence will spur advances into the research, diagnosis, and treatment of this important organism.

7:45	SESSION VII: ORAL ABSTRACTS: MICROBES ON THE MUCOSA	
SVII-1	Electronic Nicotine Delivery Systems and the Oral Microbiome: An Integrated–Omics Analysis	30
	Ganesan, S.M.;* Dabdoub. S.M.; Pamulapati, S.; Kumar, P.S.	
SVII-2	Computational Analysis of Disease-Associated Functional Shifts in the Periodontal Microbiome	31
	Dabdoub, S.M.;* Ganesan, S.M.; Kumar, P.S.	
SVII-3	Effects of Pregnancy and Smoking on the Subgingival Microbiome <i>Paropkari, A.D.;</i> * <i>Leblebicioglu, B.; Christian, L.M.; Kumar, P.S.</i>	32
SVII-4	Galectins in the Distal Reproductive Tract: A Novel Mechanism of Anaerobe Synergisms	33
	Fichorova, R.N.;* DeLong, A.K.; Cu-Uvin, S.; King, C.C.; Jamieson, D.J.; Klein, R.S.; Sobel, J.D.; Vlahov, D.; Yamamoto, H.S.; Mayer, K.H.	

SVII-1 SVII-2

ELECTRONIC NICOTINE DELIVERY SYSTEMS AND THE ORAL MICROBIOME: AN INTEGRATED-OMICS ANALYSIS

Ganesan, S.M.;* Dabdoub. S.M.; Pamulapati, S.; Kumar, P.S. Division of Periodontics, College of Dentistry, The Ohio State University, Columbus, OH USA

Introduction: 20.4 million individuals (2.5-million children) use Electronic Nicotine Delivery Systems (ENDS). Like cigarette smoke, the initial point of contact of ENDS, and the first-affected system by this agent, is the oral cavity. We have earlier shown that tobacco smoking leads to commensal-depleted, pathogen-enriched oral biofilms, thus increasing susceptibility to periodontitis. Hence, the purpose of the present study was to assess the effects of ENDS on the oral microbiome.

Methods: Subgingival plaque samples were collected from 20 ENDS-only users, 20 cigarette-smokers, 20 dual-users, 20 former cigarette-smokers current ENDS-users, and 20 controls with no oral/systemic disease, pregnancy, and recent antibiotic use. Whole genome shotgun sequencing and metagenomics were used to evaluate functional potential. The findings were validated by testing the effects of nicotine and smoke-exposure on biofilm gene expression on an *in vitro* biofilm model using RNA-Seq. Biofilm structure was evaluated using confocal microscopy.

Results: Campylobacter, Granulicatella, Leptotrichia, Neisseria, and Porphyromonas were significantly more abundant in ENDS-only users. ENDS-only users showed a higher abundance (≥ 2-fold) of virulence, stress response, membrane transport, motility, chemotaxis & iron acquisition genes when compared to both controls and smokers. Former smokers and dualusers exhibited significantly more virulence-related genes when compared to smokers. These changes in functional potential were also evident at the transcriptional level. Commensal biofilms showed more transcriptional changes in response to nicotine and smoke exposure when compared to pathogen-rich biofilms. Nicotine and smoke triggered different genes belonging to the same functional groups, implying alternate pathways in virulence expression. Nicotine exposure also altered biofilm size and structure when compared to controls and smoke exposure.

Conclusions: ENDS users have a virulence rich microbiome, and nicotine exposure leads to upregulation of virulence factors in health-compatible biofilms *in vitro*, suggesting that ENDS may not be a safe aid for smoking cessation.

COMPUTATIONAL ANALYSIS OF DISEASE-ASSOCIATED FUNCTIONAL SHIFTS IN THE PERIODONTAL MICROBIOME

Dabdoub, S.M.;* Ganesan, S.M.; Kumar, P.S. Division of Periodontics, College of Dentistry, The Ohio State University, Columbus, OH USA

Purpose: It is well known that periodontitis, a polymicrobial disease that destroys tooth-supporting structures, is taxonomically heterogenous. Yet, little is known about the functional idiosyncrasies of this ecosystem. We examined 73 microbial assemblages from 25 individuals with generalized chronic periodontitis (25 deep and 23 shallow-site samples) and 25 periodontally healthy individuals using comparative metagenomics. Core metabolic networks were computed, and abundances of functional genes examined within this framework.

Methods: 22.65 million whole-genome sequences (Illumina) were processed with the MG-RAST pipeline for functional characterization. Significant differential abundance was determined using DESeq2, network analysis with bipartite/R, and additional analysis and visualization tools were developed in Python (available on GitHub: PyMGRAST).

Results: Over two-thirds of the core metagenome, especially energy metabolism, stress response, iron transport, metal and antibiotic resistance, flagella, and LPS, diverged significantly between health and disease (deep and shallow sites). Virulence-enhancing functional synergisms were observed in disease between the virome, archeome, and bacteriome. Bacteria in health functioned as generalists, with all species contributing equally to functional needs, while specialists dominated disease, with each species contributing a defined set of functions. Even though the communities were phylogenetically heterogeneous at both subject and site levels, they were functionally congruent. Several genes demonstrated robust discriminating power between health and disease.

Conclusions: The periodontal microbiome shifts from an energy efficient ecosystem in health to a highly entropic system in disease. Global functional dysbiosis is seen in disease; with every site capable of initiating a pro-inflammatory host response. Importantly, shallow sites in individuals with disease appear to be at greater risk for harm than previously believed. The identification of taxonomically idiosyncratic but functionally similar communities supports a gene-centric rather than a species-centric theory of disease causation.

EFFECTS OF PREGNANCY AND SMOKING ON THE SUBGINGIVAL MICROBIOME

Paropkari, A.D.;*1 Leblebicioglu, B.;1 Christian, L.M.;2 Kumar, P.S.¹ Division of Periodontology, College of Dentistry, The Ohio State University, Columbus, OH USA

²The Institute for Behavioral Medicine Research, The Ohio State University Medical Center, Columbus, OH USA

In United States, more than 10% of the 21.1 million women smokers continue smoking during their pregnancy. The present study examines the effects both pregnancy and smoking on the subgingival microbiome.

Subgingival plaque samples were collected from 44 systemically and periodontally healthy non-pregnant non-smokers (control), smokers, pregnant and pregnant smokers. DNA was isolated, amplified and sequenced using 454 pyrotag sequencing. 331601 classifiable sequences were compared against GreenGenes for bacterial identification using the QIIME and PhyloToAST pipelines. UniFrac distances were compared using community ordination methods. Network graphs were computed to examine bacterial co-occurrences.

Linear Discriminant Analysis demonstrated statistically significant separation (p< 0.001, MANOVA/Wilks' Lambda) among all groups. While there were no differences in alpha diversity among groups, they were significantly different in beta diversity. Species belonging to genera Acidovorax, Acinetobacter, Aggregatibacter, Desulfobulbus, Fusobacterium, Johnsonella, Klebsiella, Methylobacterium, Neisseria, Porphyromonas, Pseudomonas, Tannerella, and Treponema were significantly different (p<0.05, Tukey-HSD) among groups. Species belonging to Acidovorax, Dermabacter, Diaphorobacter, Klebsiella, Methyloversatilis, and Sphingobium predominated the microbiomes of pregnant group, while Abiotrophia, Aggregatibacter, Cardiobacterium, Haemophilus, Neisseria, and Porphyromonas were predominant in controls. The greatest numbers of significant bacterial cooccurrences were observed in pregnant group, with the dominant members of the community anchoring these networks. Prevotella demonstrated a strong and significant negative co-occurrence with several other genera in pregnant group.

Both smoking and pregnancy alter the composition of the subgingival microbiome in different ways. The effects of the two factors together are significantly different from either factor alone. Further studies are required to explore the resilience of the microbiome post parturition.

GALECTINS IN THE DISTAL REPRODUCTIVE TRACT— A NOVEL MECHANISM OF ANAEROBE SYNERGISMS

Fichorova, R.N.;*1 DeLong, A.K.;² Cu-Uvin, S.;³ King, C.C.;⁴ Jamieson, D.J.;⁵ Klein, R.S.;⁴ Sobel, J.D.;⁵ Vlahov, D.;⁵ Yamamoto, H.S.;¹ Mayer, K.H.^{9,10}, for the HERS investigators

¹Laboratory of Genital Tract Biology, Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Brigham and Women's Hospital, Boston, MA USA

²Center for Statistical Sciences, School of Public Health, Brown University, Providence, RI USA

³Division of Infectious Diseases, Department of Obstetrics and Gynecology, Brown University, The Miriam Hospital, Providence, RI USA

⁴Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA USA

⁵Division of Infectious Diseases, Department of Medicine, The Icahn School of Medicine at Mt. Sinai, New York, NY USA

⁶Division of Infectious Diseases, School of Medicine, Wayne State University, Detroit, MI USA

⁷Department of Community Health Systems, School of Nursing, University of California at San Francisco, San Francisco, CA USA

9The Fenway Institute, Fenway Health, Boston, MA USA

¹⁰Infectious Disease Division, Department of Medicine, Beth Israel Deaconess Hospital, Harvard Medical School, Boston, MA USA

The most common non-viral sexually transmitted pathogen, the protozoan anaerobe T. vaginalis (TV) frequently co-occurs with bacterial vaginosis. We explored galectins as underlying mechanism for synergism in evading host immunity. Immunity was assessed in cervicovaginal samples from 316 participants in the HIV Epidemiology Research Study at 223 TV-positive visits following a TV-negative visit and 223 visits from controls who remained TV-negative throughout the 7-year study, matched by age, race, HIV, and BV status. We assessed how bacterial vaginosis, HIV, HSV-2, and HPV modify vaginal immunity in women with trichomoniasis. Odds ratios (OR, 95% CI) for incident TV with each log10 unit higher inflammation were calculated using conditional logistic regression. The highest odds for incident TV were associated with galectin-9 (OR=2.91, 95% CI: 2.10-4.03). Among the TV positive women, galectin-9 levels were higher when women had BV and abnormal Nugent scores. In contrast, higher levels of galectin-3 were associated with lowest odds of incident TV among BV-negative women (0.03, 0.00-0.48). Among the TV-positive visits, those with BV had lower levels of galectin-3. These clinical data were in agreement with our experimental findings of causality and support the conclusion that galectins impart the proinflammatory synergisms between protozoan, viral and bacterial pathogens and could be targeted to avoid the breakdown of the vaginal immune barrier.



Wedne	sday, July 13, 2016 Oral Anaero	Oral Anaerobes	
905	SESSION VIII: ESCMID-ASA JOINT SYMPOSIUM: FOCUS ON ORAL ANAEROBES		
SVIII-1	Highlights on Anaerobe Research in ESCMID Nagy, E.*	36	
SVIII-2	Relationship Between Methanogenic Archaea and Subgingival Microbial Complexes in Human Periodontitis Conrads, G.;* Horz, H.P.	37	
SVIII-3	Disruption of Immune Homeostasis in Pathogen Induced Vascular Inflammation Genco, C.A.*	38	
SVIII-4	Diabetes and the Oral Microbiome	39	

Kumar, P.*

34

HIGHLIGHTS ON ANAEROBE RESEARCH IN ESCMID

Nagy, E.*

Institute of Clinical Microbiology University of Szeged, Szeged, Hungary ESCMID Study Group on Anaerobic Infections

The main goals of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) are managing infections and promoting science. This is also true for infections caused by anaerobic bacteria. Since the 1960s and 1970s an increasing interest developed, not only in the U.S., but also in Europe on the different methods how the isolation and identification of the anaerobic bacteria can be improved in routine and research laboratories. Despite of the developments to provide appropriate culture condition for strict anaerobic bacteria still in many cases, anaerobic infections were and still are treated on the empiric bases. Since the 1990s ESCMID through its Study Group on Antibiotic Resistance of Anaerobic Bacteria (ESGARAB) supported Europewide surveillances on antibiotic resistance among the most common anaerobic pathogens such as the Bacteroides, Prevotella, Gram-positive cocci, etc. The steady increase of resistance to ampicillin, cefoxitin, erythromycin, clindamycin, and tetracyclin was confirmed similar to the situation in other parts of the world. However, less frequent resistance mechanisms were also confirmed among Bacteroides strains involving amoxicillin/clavulanic acid, piperacillin/ tazobactam, moxifloxacin, and in less extent metronidazole and carbapenems. It became evident that the antibiotic susceptibility testing is needed and to set clinically valid breakpoints is a must for further developments in anaerobic bacteriology. EUCAST breakpoints for the most frequently isolated anaerobes and the first and ongoing developments on the use of disc diffusion method for the "rapid growing anaerobes" is still discussed in the EUCAST. Several studies supported by ESGARAB and by the renewed ESCMID Study Group on Anaerobic Infections (ESGAI) are carried out evaluating the presence of different resistance genes and their expression among clinical isolates, as well as among normal flora isolates of Bacteroides and Parabacteroides. The arrival of MALDI-TOF MS had an immediate, significant impact on anaerobic microbiology in Europe. After the early years of the database developments, many laboratories reported extensive studies on the characterisation of anaerobic species using MALDI-TOF MS and its implementation in clinical laboratories has been transformative. The joint efforts of ESGAI and the ESCMID Study Group on Epidemiological Markers (ESGEM) together with several European anaerobic reference laboratories, led to the announcement of the ENRIA project (European Network for the Rapid Identification of Anaerobes), to improve the database of the Bruker Biotyper, by including the MSPs of several clinical anaerobic isolates. As a European approach, the MALDI-TOF MS-based differentiation of the cfiA gene harbouring Bacteroides strains was introduced in the system and several ongoing studies try to find the place of the MS-based typing of *Propionibacterium* acnes in clinical routine laboratories as well. During the last ECCMID in Amsterdam, as well as during the present ASA congress, numerous posters show the achievements of ESCMID members in the field of anaerobe research.

RELATIONSHIP BETWEEN METHANOGENIC ARCHAEA AND SUBGINGIVAL MICROBIAL COMPLEXES IN HUMAN PERIODONTITIS

SVIII-2

Conrads, G.;* Horz, H.P.

Division of Oral Microbiology and Immunology and Department of Medical Microbiology, RWTH Aachen University Hospital, Aachen, Germany

Archaea, organisms that make up the third domain of cellular life are members of the human oral microflora. They are strikingly less diverse than oral *Bacteria* and appear to be relatively rare with respect to their numerical abundance. In the essence, only one distinct cultivated representative of archaea, namely the methanogenic *Methanobrevibacter oralis*, is pre-dominating in the mouth, though a few other species including *Thermoplasmatales* have recently been described. Since they have been exclusively found in association with oral infections such as periodontitis and apical periodontitis and given their unique physiology and energy metabolism, it is highly plausible that they are more than just secondary colonizers of infected areas, but instead are actively involved in the overall poly-microbial infectious process. Conversely, it is a highly challenging task to clearly demonstrate their possible active participation mostly due to the difficulty to grow them in routine microbiology laboratories.

This lecture points out the importance of understanding the medical impact of methanogens in general and aims at devising strategies for elucidating the true function of archaea in the oral ecosystem. To this end, we compared the amounts of methanogenic archaea with ten of the most important periodontal pathogens in 125 clinical periodontal samples.

Correlation analysis suggests that the support of the periodontitis-associated bacterial consortium by methanogenic archaea may be driven through direct or indirect interactions with *Prevotella intermedia*, a species known for its broad substrate range for fermentation (i.e. growth on carbohydrates and proteins). Enlarged metabolic versatility may lead to the production of a broader range of short chain fatty acids required for interspecies hydrogen transfer thereby providing more opportunities for *P. intermedia* to engage in syntrophic interactions with methanogens. Hence, metabolic versatility may be the clue that would explain the observed correlation between *P. intermedia* and *M. oralis*.

SVIII-3 SVIII-4

DISRUPTION OF IMMUNE HOMEOSTASIS IN PATHOGEN INDUCED VASCULAR INFLAMMATION

Genco, C.A.*

Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine, Boston, MA USA

Unresolved inflammation is undoubtedly a contributing factor in progressive inflammatory diseases. However, due to the predominant role of inflammation in clearing infections, there is a fine balance between activation of immunity and unresolved inflammation. Several successful pathogens have evolved mechanisms to evade host defense, resulting in the establishment of persistent infection and chronic inflammation. One such pathogen, *Porphyromonas gingivalis*, induces chronic low-grade inflammation associated with systemic inflammatory diseases in humans including diabetes, pre-term birth, and cardiovascular disease. Animal models have validated human studies and our studies have reported novel and substantial data that provide new insights into pathogen- and Toll-like receptor (TLR)-specific molecular pathways linking oral infection with P. gingivalis to site-specific vascular inflammation. P. gingivalis expresses a number of outer membrane components that interact at TLR2 and TLR4. Significantly, P. gingivalis evades TLR4 signaling through expression of divergent lipid A structures that function as antagonists, weak agonists, or are non-activating. We utilized a series of *P. gingivalis* lipid A mutants to demonstrate that antagonistic lipid A structures enable the pathogen to evade TLR4 detection by monocytic cells resulting in increased site-specific vascular inflammation. Importantly, a P. gingivalis strain producing agonistic lipid A augmented levels of proinflammatory mediators and activated the inflammasome, resulting in decreased bacterial survival and diminished sitespecific vascular inflammation. We also demonstrated that the expression of immune evasive lipid A species (antagonist/non-activating) enables this bacterium to evade TRIF-dependent expression of IFNβ in dendritic cells. Expression of P. gingivalis immune evasive lipid A species was associated with an uncoupling of proinflammatory cytokine production from dendritic maturation, as measured by up-regulation of co-stimulatory molecules that are crucial for naïve T cell activation. Our studies support a mechanism by which pathogen stimulation results in the dysregulation of the innate and adaptive response that promotes low-grade chronic inflammation. Findings from these studies may have implications for the mechanisms underlying pathogen persistence and systemic immune pathologies.

DIABETES AND THE ORAL MICROBIOME

Kumar, P.S.* Ohio State University, Columbus, OH USA

Although smoking and diabetes have long been established as the only risk factors for periodontitis, their individual and synergistic impacts on the periodontal microbiome have not been well studied. The present investigation used 1.7 million 16S pyrotag sequences to assess subgingival bacterial biodiversity and co-occurrence patterns in non-smoking normoglycemic individuals (controls), smokers, diabetics, and diabetic smokers; and to identify ecological shifts during transition from health to disease in these high-risk individuals. Smokers, diabetics and diabetic smokers with periodontitis demonstrated less complex communities than controls. In contrast to diabetics and diabetic smokers, a robust core microbiome was observed in smokers, and differences between individuals were attributable largely to the 'rare biosphere'. Anaerobic organisms predominated in smokers, whereas diabetics and diabetic smokers were enriched for facultative species. In smokers, bacterial co-occurrence was sparse and predominantly congeneric, while robust inter-generic networks were observed in diabetics and diabetic smokers. Lastly, the microbial signatures of both periodontally healthy diabetics and smokers were highly aligned with disease; in smokers, there were few differences in community membership between periodontal health and disease, whereas in diabetics, periodontal health and disease demonstrated significantly different taxonomical profiles. The microbial profile of periodontitis in diabetic smokers was more similar to diabetics than to smokers, however differences in community membership, structure and co-occurrence were still apparent between diabetics and smokers. Thus, this study underscores the need for personalized risk assessment strategies in the management of multifactorial diseases.



Wednesday, July 13, 2016Taxonomy and the Anaerobes1100SESSION IX: WHAT'S IN A NAME: TAXONOMY AND THE ANAEROBESSIX-1The Taxonomy of the Genus Clostridium: Current Status and Future Perspectives for the Clinical Community 42
Lawson, P.A.;* Rainey, F.A.SIX-2Reconciling Phenotype vs. Genotype in the Taxonomic Classification of Anaerobes

Bernard, K.A.*

THE TAXONOMY OF THE GENUS *CLOSTRIDIUM*: CURRENT STATUS AND FUTURE PERSPECTIVES FOR THE CLINICAL COMMUNITY

Lawson, P.A.;*1 Rainey, F.A.2

- ¹Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK USA
- ²Department of Biological Sciences, University of Alaska Anchorage, Anchorage, AK USA

The genus Clostridium was proposed in 1880 by Prazmowski with the type species C. butyricum, subsequently the genus became a general depository for Gram-positive staining, sporeforming, anaerobic organisms. Consequently, the genus as presently constituted is phylogenetically and phenotypically incoherent; biochemical, chemical and molecular data indicate that the genus comprises a collection of very heterogeneous species. Presently, there are 238 validly published Clostridium species and subspecies with frequent new additions. Numerous phylogenetic studies based principally on sequencing of the 16S rRNA gene, but supported by protein signatures, indicate that the genus Clostridium should be restricted to Clostridium rRNA cluster I as Clostridium sensu stricto. Despite this evidence, authors continue to name organisms as Clostridium thus further perpetuating the confusion associated with the taxonomy of this important group of organisms. Specific issues concerning the C. botulinum complex, C. difficile and other clinically relevant organisms will be addressed. A discussion will be presented on the recent proposal to restrict Clostridium to the type species Clostridium butyricum and relatives and the implications for the scientific and clinical community.

RECONCILING PHENOTYPE VS. GENOTYPE IN THE TAXONOMIC CLASSIFICATION OF ANAEROBES

Bernard, K.A.*1

¹National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB Canada

The accurate and timely identification of anaerobes has always been a challenging one. Phenotypic characterization using 'spot tests', an extensive panel of substrates or a rapid strip panel, is still performed widely as the sole means of identification in many laboratories. The use of 16S rRNA gene sequencing beginning in the 1990s for both identification and serving as the backbone for the taxonomic 'definition' of genera and species, is widely used in laboratories that routinely employ complex test methods. The use of 16S analyses coupled to biochemical and chemotaxonomic characteristics has helped to improve taxonomic 'precision' for anaerobes, when coupled to globally accepted 'Rules' for sp nov descriptions, as outlined in the International Code of Nomenclature for Prokaryotes [IJSEM, in press] and endorsed by the International Committee for the Systematics of Prokaryotes (http://www.the-icsp.org/). The study of anaerobes is now shifting towards the routine study of whole genome sequences (WGS). Many laboratories now use molecular methods almost exclusively when identification of pathogens as required, whether by analysis of single targets like 16S genes or by WGS. This has given rise to some confusing trends, which will be elaborated on in this talk. Clinicians must now grapple with evolving, complex taxonomic issues such as (1) authors publishing a brief overview of species which are never 'validated', (2) proposed nomenclature changes that were published but never validated and ended up as entrenched among NCBI's taxonomy tools, and (3) the use of brief announcements to introduce newly published WGS as 'species nova', but which are never validated or, in some cases, are validated but do not provide few traditional descriptors, such as colony morphology, Gram stain arrangement, reactions to any biochemical tests, or provide any chemotaxonomic features. This trend is setting a possible precedent for the creation of a parallel system for naming or characterizing new taxa, which lie outside of the 'Rules" outlined in the Code of Nomenclature.



Wednesday, July 13, 2016 **Bacterial Replacement Therapy 1400** SESSION X: BACTERIAL REPLACEMENT THERAPY **INCLUDING FECAL MICROBIOME TRANSPLANTS** SX-1 Microbiota and Mental Health: Hype or Hope? 46 Foster, J.A.* SX-2 Fecal Microbiota Transplant for Ulcerative Colitis 47 Surette, M.G.* SX-3 Fecal Microbiota Transplant in Clinical Practice 48 Kelly, C.* SX-4 Feasibility of a Room Temperature Stable, Orally Delivered Microbiota Capsule for the Prevention of Recurrent Clostridium difficile Infection 49 Garg, S.;* Brown, B.; Erickson, J.; Burroughs, M. SX-5 Host-Specific Fecal Microbiota Transplantation is More Effective in Treating Recurrent Clostridium difficile Infection in a Murine Model 50 Seekatz, A.M.;* Theriot, C.M.; Vendrov, K.C.; Young, V.B. SX-6 SYN-004: A Pioneering Therapeutic to Protect the Microbiome

Connelly, S.;* Bristol, J.A.; Hubert, S.; Hasan, N.A.; Subramanian, P.;

from Antibiotic-Mediated Damage

Furlan-Freguia, C.; Sliman, J.; Kaleko, M.

51

MICROBIOTA AND MENTAL HEALTH: HYPE OR HOPE?

Foster, J.A.*

Department of Psychiatry & Behavioural Neurosciences, McMaster University, Hamilton, ON Canada

Excitement has been generated in mental health research by recent findings from animal and clinical studies demonstrating an important role for gut microbiota in brain function and behaviour. This emerging area of research has researchers and the public starting to take notice of microbes and the mind. Scientists have established a link between gut bacteria and anxietylike behaviours in animal models and with emotional brain regions in healthy people. Similar observations in rodents and humans related to microbiota-brain influence on stress circuitry and emotional behaviours suggest that aspects of this process are conserved and can be studied in animal models. Our initial results revealed that germ-free mice showed reduced anxiety-like behaviour in the elevated plus maze, a well-established behavioural test that examines approach and avoidance behaviour in mice, in comparison to conventionally housed mice. Additional work by our group and others to date suggest that microbiota influence brain structure, gene expression of stress-related and plasticity-related genes, stress-reactivity, and behaviour. Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Several studies using animal models and in clinical populations that considers the beneficial effects of probiotics. Advances in the field of microbiota-brain research that relate to neuroscience and psychiatry and whether probiotics have potential in treatment of psychiatric illnesses including anxiety and depression will be considered.

FECAL MICROBIOTA TRANSPLANT FOR ULCERATIVE COLITIS

Surette, M.G.* McMaster University, Hamilton, ON Canada

Ulcerative colitis (UC) is a chronic gastrointestinal disease of the colon characterized by severe inflammation and ulceration of the colon. It is hypothesized that an altered microbial community in the colon drives the inflammation and that correction of the microbial dysbiosis could restore the colon to a normal state. Fecal microbial transplantation (FMT), using healthy donor bacteria as a treatment to correct the dysbiosis, has shown remarkable efficacy in the treatment of recurrent C. difficile infection and has been proposed as a therapy for a wide variety of gastrointestinal diseases. We recently completed a large randomized placebo controlled trial of FMT in treatment of moderate to severe UC and demonstrated FMT is effective at inducing complete remission in ~ 25% of patients (Moayyedi, et. al. 2015 Gastro. 49:102-109). Here, we present the analysis of stool and mucosal biopsy microbiomes from this trial and a case study of one patient followed over 14 months. Two findings stand out from our analysis. First, we observe a significant donor effect, with one donor accounting for >75% of successful treatments. Secondly, it is surprisingly difficult to demonstrate consistent transfer of donor bacteria to the recipient. This raises the question whether engraftment is necessary for successful treatment of UC and underscores the need for a mechanistic understanding of FMT. As a therapy FMT is not without risks to the patient, and increasing the efficacy of FMT in treatment of GI disease and reducing potential risks to patients will benefit from a more thorough understanding of the microbiological changes associated with UC and remission.

FECAL MICROBIOTA TRANSPLANT IN CLINICAL PRACTICE

Kelly, C.* Brown University, Providence, RI USA

Clostridium difficile is one of the most basic models of how disruptions in the gut microbiota can result in disease. The incidence of *C. difficile* infection (CDI) has increased rapidly in the past decade with cases that are more severe and often recur after treatment with standard antimicrobial therapies. Fecal microbiota transplantation (FMT) has emerged as a promising method to treat patients suffering from recurrent, refractory, or severe CDI. FMT involves administering fecal material from a healthy individual (donor) into a recipient to promote colonization with beneficial components of normal microbiota. Multiple case series and several recent randomized controlled clinical trials have demonstrated the efficacy and safety of FMT. FMT is rapidly evolving in several directions. Commercialized preparations of minimally modified stool and defined microbiota formulations, in which the species responsible for therapeutic effects are isolated and delivered, have been effective in human trials. Stool banks have centralized the donor screening process offering convenience and cost savings and now supply donor material to clinicians and researchers. Interestingly, the US Food and Drug Administration (FDA) has determined that FMT constitutes a "drug" and a "biological product" and regulatory oversight of this therapy is required.

This lecture will review the evidence supporting the efficacy and safety of FMT for the treatment of *C. difficile* infections and discuss suspected mechanisms. It will focus on FMT methods, including donor selection and testing, preparation of FMT material, administration protocols and discuss challenges to widespread implementation. Additionally, we will discuss the future of FMT and introduce regulatory issues which have emerged and how they impact the use of FMT for CDI and other conditions.

FEASIBILITY OF A ROOM TEMPERATURE STABLE, ORALLY DELIVERED MICROBIOTA CAPSULE FOR THE PREVENTION OF RECURRENT *CLOSTRIDIUM DIFFICILE* INFECTION

Garg, S.,* Brown, B.; Erickson, J.; Burroughs, M. Rebiotix Inc., Roseville, MN USA

Background: Disruption of the intestinal microbiota, by factors such as antibiotic use, has been implicated in *Clostridium difficile* infection (CDI). CDI has been treated successfully with microbiota-based therapies that have been delivered into the intestinal tract via nasogastric tube, colonoscopy, and enema. A room-temperature stable, orally-delivered microbiota capsule may provide a number of advantages in terms of patient dosing and therapy access.

Objective: To characterize the bacterial composition of multiple formulations of a Microbiota Restoration Therapy (MRT) for delivery in an oral dosage form after processing and upon storage.

Methods: Multiple powders were manufactured from full spectrum human intestinal microbiota; filled into capsules and then studied after processing and storage at pharmaceutically-relevant conditions. Standard plating, propidium monoazide (PMA)-quantitative polymerase chain reaction (qPCR) and 16s rRNA gene sequencing methods were employed to determine the amount and type of viable bacteria present in the MRT capsule.

Results: The quantity of viable bacteria in multiple capsule formulations was maintained at 5°C, 25°C, and 35°C for at least 3 months. The 16s rRNA sequencing data indicate that the encapsulated product maintained bacterial alpha-diversity across multiple process technologies and formulations.

Conclusion: Process and formulation parameters that enable production of a room temperature-stable MRT capsule have been identified. Formulations containing bacterial concentrations high enough to support a capsule regimen for clinical testing have been achieved.

HOST-SPECIFIC FECAL MICROBIOTA TRANSPLANTATION IS MORE EFFECTIVE IN TREATING RECURRENT CLOSTRIDIUM DIFFICILE INFECTION IN A MURINE MODEL

Seekatz, A.M.;*1 Theriot, C.M.;² Vendrov, K.C.;¹ Young, V.B.¹ Internal Medicine, Division of Infectious Diseases, University of Michigan, Ann Arbor, MI USA

²College of Veterinary Medicine, Department of Population Health and Pathobiology, North Carolina State University, Raleigh, NC USA

Recurrent Clostridium difficile infection (CDI) occurs in 20-30% of patients following successful treatment of an initial episode of CDI. Defects in the gut microbiota, the indigenous bacterial community within the gastrointestinal tract, are hypothesized to contribute to the development of recurrence. Fecal microbiota transplantation (FMT) has emerged as an effective treatment for recurrent CDI, and its success correlates to an increase in microbes associated with a healthy gut community. We previously characterized a murine model of recurrent CDI, and observed that mouse feces (mFMT) are sufficient to clear C. difficile. To identify potential bacterial members or community structures in humans that promote recovery from recurrent CDI, we treated recurrent animals with feces from a) healthy human donors (hdFMT) and b) pre-FMT human recipients (hrFMT) from previously successful FMT human donor-recipient pairs. While mFMT from multiple mouse colonies was successful in clearing C. difficile, FMT from human sources was significantly less effective. We used 16S rRNA analysis to identify bacterial taxa associated with *C. difficile* clearance. Interestingly, we observed significant overlap of operational taxonomic units (OTUs) between mFMT- and hdFMT-treated animals, regardless of disease outcome. In particular, both FMT treatments resulted in an increase in several Bacteroidetes, such as the Bacteroides and Porphyromonadaceae groups, and Firmicutes, including several *Clostridiales* and *Lachnospiraceae* groups. These results emphasize the importance of the development of specific host-microbe interactions and suggest that host specificity to gut microbial communities is important in the dynamics of *C. difficile* colonization. Future studies that incorporate both human and murine models to identify beneficial bacteria for human health should consider how host specificity potentially impacts outcome.

SYN-004: A PIONEERING THERAPEUTIC TO PROTECT THE MICROBIOME FROM ANTIBIOTIC-MEDIATED DAMAGE

Connelly, S.;*1 Bristol, J.A.;1 Hubert, S.;1 Hasan, N.A.;2 Subramanian, P.;2 Furlan-Freguia, C.;1 Sliman, J.;1 Kaleko, M.1 Synthetic Biologics, Inc., Rockville, MD USA CosmosID, Inc., Rockville, MD USA

SYN-004 is a clinical-stage, oral b-lactamase intended to preserve the gut microbiome by inactivating residual antibiotics (abx) in the intestine. Abxmediated microbiome disruption can lead to abx-associated diarrhea (AAD) and C. difficile infection (CDI). A Phase 2b clinical study is in progress to assess SYN-004-mediated prevention of AAD and CDI in patients receiving IV ceftriaxone (CRO). The purpose of the preclinical study described here was to determine if SYN-004 efficacy could be expanded to include oral abx. SYN-004 was manufactured in E. coli and formulated into entericcoated pellets that release enzyme in the duodenum (at pH >5.5). SYN-004 was tested in normal piglets (~20 kg, n=5 per cohort) treated with IV CRO (50 mg/kg QD for 7 days) or oral amoxicillin (AMX; 20 mg/kg BID for 7 days). Whole genome shotgun sequence analyses of pig fecal DNA and quantification of serum abx levels were performed. For both CRO and AMX, serum levels were similar +/- SYN-004 indicating that SYN-004 did not alter systemic abx levels. Remarkably, for oral AMX, these data demonstrate that SYN-004 did not degrade the abx in the GI tract prior to absorption. Microbiome analyses showed that SYN-004 prevented both CRO- and AMX-mediated dysbiosis including protection of species diversity, further supporting its clinical potential in humans. Likelihood ratio tests, performed using a parameterization of the Dirichlet-Multinomial distribution, revealed that the pre- and post-treatment microbiomes were significantly different in the abx-alone cohorts (p<0.001), while they were not different in the abx+SYN-004 cohorts (p>0.1). As SYN-004 did not affect the absorption of oral AMX while protecting the microbiome, these data suggest that SYN-004 is released into the GI tract at a point distal to the absorption of AMX but still proximal enough to degrade residual AMX. Therefore, SYN-004 has the potential to become the first therapy designed to protect the microbiome from abx-mediated damage and prevent AAD and CDI. In addition, the data support further development of SYN-004 for use with oral abx.



Wedne	esday, July 13, 2016	Genital Tract Anaerobe	: 5
1610		ONS OF GENITAL TRACT S & RELATIONSHIP TO HUMAN	Γ
SXI-1	Cultivation of Novel Anaerobe Hillier, S.L.*	s from the Vagina 5	4
SXI-2	Comparative Genomics of Vag Metabolites <i>in vivo</i> and <i>in vitro</i> <i>Srinivasan</i> , <i>S</i> .*	inal Anaerobes and Linkage to 5	5
SXI-3	Cultivation of Vaginal Anaerok Associated Models <i>Pyles, R.B.</i> *	es in Epithelial Cells and 5	6
SXI-4	Bacterial Species Colonizing th Are Not Associated with Race	e Vagina of Healthy Women 5	7

Beamer, M.A.;* Meyn, L.A.; Bunge, K.; Hillier S.L.

57

CULTIVATION OF NOVEL ANAEROBES FROM THE VAGINA

Hillier, S.L.*

Magee-Womens Research Institute, University of Pittsburg, Pittsburgh, PA USA

Cultivation based methods for characterization of the vaginal microbiota have revealed a broad diversity of microorganisms, many of which have not been classified taxonomically. We have recovered over 300 unique bacterial species from vaginal samples, with 20% of these representing novel organisms which may have previously been considered uncultivable. These have included Megasphaera phylotypes 1-3, BVAB2, BVAB3 (Mageeibacillus indolicus), and six novel Prevotella species. There have been more than 10 novel anaerobic gram negative rods and more than 15 novel anaerobic gram positive rods recovered to date from vaginal samples that cannot be classified even to the genus level. To recover these novel species, vaginal fluid is collected on swab samples and transported within 24 hours in an anaerobic transporter. In the lab, vaginal fluid is eluted from the swabs, serially diluted and inoculated onto 27 different pieces of culture media. Colonies are visualized under a dissecting microscope and subcultured to purity. After DNA extraction, 16S rDNA gene sequencing is used for identification. Even using optimized cultivation based methods, Megasphaera phylotypes are much less frequently recovered using cultivation vs quantitative PCR assays from the same samples. However, obtaining pure culture isolates of these microorganisms has allowed antimicrobial susceptibility testing, full sequencing, and phenotypic characterization. This can contribute to our ability to evaluate the role of these novel microorganisms in bacterial vaginosis. For example, Megasphaera phylotypes are highly associated with bacterial vaginosis, and treatment studies evaluating the impact of metronidazole therapy on Megsphaera phylotypes have demonstrated their decrease following successful treatment and persistence among those women having treatment failure. To date, 123 isolates of Megasphaera phylotypes 1-3 have been tested for susceptibility to metronidazole, tinidazole, and clindamycin and all have MICs of <1 ug/mL. Thus, antimicrobial resistance likely does not account for the persistence of this microorganism among women who experience treatment failure. A combination of cultivation and cultivation independent methods will be required for full characterization of the diversity of the vaginal microbiome.

COMPARATIVE GENOMICS OF VAGINAL ANAEROBES AND LINKAGE TO METABOLITES IN VIVO AND IN VITRO

Srinivasan, S.*

Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA USA

The human vaginal microbiome has the potential to impact the health of women, their sex partners, and their neonates. The dysbiotic condition bacterial vaginosis (BV) has been associated with numerous adverse health outcomes such as cervicitis, acquisition of HIV, and preterm birth. The etiology and pathogenesis of BV are poorly understood. A hallmark of BV is the transformation of the bacterial community from primarily *Lactobacillus* species to diverse anaerobic bacteria, with a corresponding change in the composition of metabolites in vaginal fluid. These metabolites impact at least three of four clinical criteria used in the diagnosis of BV including elevated pH, presence of an amine odor, and presence of abnormal vaginal discharge. Little is known about the genomic and metabolic capacity of BV-associated bacteria, as many bacteria are fastidious and have eluded laboratory propagation. Our recent intensive cultivation efforts as part of the Human Microbiome Project have resulted in the isolation of a large collection of BV-associated bacteria. The bacterial isolates were categorized into four groups including novel strains with <98% 16S rRNA sequence identity to validly described species such as *Dialister* sp. Type 2 and Eggerthella sp. Type 1, closely related species within a genus, bacteria previously isolated from body sites other than the vagina, and known bacteria formerly isolated from the vagina. DNA from representative isolates has been submitted for whole genome sequencing, and the cultivated bacteria will facilitate experimental manipulation of these organisms in the laboratory to better understand microbial interactions and pathogenesis. We sought to dissect the genomic and metabolic capacity of BV-associated bacteria to help inform routine growth of these bacteria under laboratory conditions. Experimental manipulation of cultivated BV-associated bacterial isolates can be challenging as bacterial growth is often inconsistent. Using the example of the fastidious anaerobe Mageeibacillus indolicus (BVAB3), I will discuss how genomic analysis and metabolic pathway reconstruction can help predict substrates utilized by M. indolicus to facilitate reproducible propagation under *in vitro* conditions. These studies will help disentangle metabolic networks in BV, and illuminate how vaginal anaerobes interact to sustain this complex community of bacteria.

CULTIVATION OF VAGINAL ANAEROBES IN EPITHELIAL CELLS AND ASSOCIATED MODELS

Pyles, R.B.* University of Texas Medical Branch, Galveston, TX USA

The vaginal microbiome is a crucial factor in women's health and fertility. Normobiosis is associated with resistance to infection, while dysbiosis can produce symptomatic inflammation and increased susceptibility to a variety of pathogens including HIV. We have developed the first ex vivo culture system that provides reproducible cultivation of intact clinical microbiomes in the context of a reconstructed vaginal mucosa. We have used this system to study microbiomes dominated by bacteria traditionally categorized as anaerobes or aerobes that thrive in the microaerophilic environment common to the vaginal vault. Using controlled environmental alterations we have begun to address changing host conditions on the profile of the vaginal bacterial community with respect to anaerobes in the community. Finally, we have used the model to study community shifts on transcription changes associated with altered host functions including movement of vaginally applied compounds. Collectively, our data validate the utility of the model for both bacterial and host outcomes and support the continued use of this model system for prediction of human vaginal health.

BACTERIAL SPECIES COLONIZING THE VAGINA OF HEALTHY WOMEN ARE NOT ASSOCIATED WITH RACE

Beamer, M.A.;*¹ Meyn, L.A.;^{1,2} Bunge, K.;² Hillier, S.L.^{1,2}
¹Magee-Womens Research Institute
²University of Pittsburgh Department of Obstetrics, Gynecology and Reproductive Sciences, Pittsburgh, PA USA

Several studies describing the vaginal microbiome have concluded that there are significant differences in the microbiome of healthy women, based on race or ethnicity. However, many of these studies have defined healthy as lack of symptoms and have failed to exclude women having sexually transmitted diseases (STIs) or bacterial vaginosis (BV). Our objective was to compare the cultivable microbiota of black and white women, after excluding genital infection.

Vaginal swabs for Nugent score, quantitative PCR (qPCR), and culture were collected from 49 asymptomatic women aged 18-45 uninfected by *C. trachomatis*, *N. gonorrhoeae*, HIV, *T. vaginalis* and who did not meet the Amsel or Nugent criteria for BV. For qPCR, bacterial DNA from vaginal swabs was extracted, combined with specific primers targeting species and stained using SYBR green binding dye for detection (gene copies/swab). For cultivation based methods, swab eluant was serially diluted and inoculated onto media. Each colony type was subcultured and identified by sequencing a 900 basepair portion of the 16S rRNA gene. Fisher's exact test was used to compare prevalence of organisms between white and black women.

Enrollment included 33 white and 16 black women. The prevalence of selected microbiota detected by qPCR at $\geq 10^5$ was similar for white vs black women: Lactobacillus crispatus (85% v 69%, P=0.26), L. iners (79% v 94%, P=0.24), Gardnerella vaginalis (73% v 75%, P>0.99), Atopobium vaginalis (27% v 19%, P=0.73), or Megasphaera type 1 (6% v 13%, P=0.59). Using intensive culture methods, no differences in frequency were detected for any of these microorganisms. In addition, Mycoplasma hominis or Ureaplasma species (42% v 50%, P=0.76), Prevotella timonensis (9% v 25%, P=0.20), P. bivia (33% v 44%, P=0.54), and Porphyromonas uenonis (3% v 6%, P>0.99) were also similar in white vs. black women.

After excluding women having STIs and/or BV, no bacterial species were significantly different in white vs black women. Although limited by small sample size, these data suggest that reports of ethnic difference in vaginal microbiome should be interpreted with caution, unless asymptomatic infections are excluded.



58

Thursday, July 14, 2016

Clostridium difficile I

730	SESSION XII: ORAL ABSTRACTS: CLOSTRIDIUM DIFFICILE I	
SXII-1	Re-Examining the Germination Phenotypes of Several <i>Clostridium difficile</i> Strains	60
	Bhattacharjee, D.; Francis, M.B.; Ding, X.; McAllister, K.N.; Shrestha, R.; Sorg, J.A.*	
SXII-2	Clathrin-Independent Endocytosis of Clostridium difficile Toxin A Chandrasekaran, R.;* Lacy, D.B.	61
SXII-3	CdtR: [Only?] The Regulator of Binary Toxin in Clostridium difficile?	62
	Bilverstone, T.; Kinsmore, N.L.; Stevenson, E.; Minton, N.P.; Kuehne, S.A.*	
SXII-4	Leptin Signaling Alters Neutrophil Homeostasis during <i>Clostridium difficile</i> Infection	63
	Madan, R.;* Xue, J.; Petri Jr., W.A.; Deepe Jr., G.	
SXII-5	Intraspecific Competition and Adaptive Immune Responses in Protection Against Murine Clostridium difficile Infection	64
	Leslie, I.L.: Jenior, M.L.: Young, V.B.*	

59

RE-EXAMINING THE GERMINATION PHENOTYPES OF SEVERAL CLOSTRIDIUM DIFFICILE STRAINS

Bhattacharjee, D.; Francis, M.B.; Ding, X.; McAllister, K.N.; Shrestha, R.; Sorg, J.A.*

Department of Biology, Texas A&M University, College Station, TX USA

Clostridium difficile spore germination is essential for colonization and disease. The signals that initiate *C. difficile* spore germination are a combination of taurocholic acid (a bile acid) and glycine. Interestingly, the chenodeoxycholic acid-class (CDCA) bile acids competitively inhibit taurocholic acid-mediated germination suggesting that compounds that inhibit spore germination could be developed into drugs that prophylactically prevent *C. difficile* infection or reduce recurring disease. However, a recent report called into question the utility of such a strategy to prevent infection by describing C. difficile strains that germinated in the apparent absence of bile acids or germinated in the presence of the CDCA inhibitor. Because the mechanisms of *C. difficile* spore germination are beginning to be elucidated, the mechanism of germination in these particular strains could yield important information on how C. difficile spores initiate germination. Therefore, we monitored germination of these strains using optical density assays and release of DPA from the spore core during germination. In this way, we quantified the interaction of these strains with taurocholic acid and CDCA, the rates of spore germination, the release of DPA from the spore core and the abundance of the germinant receptor complex (CspC, CspB and SleC). We find that strains previously observed to germinate in the absence of taurocholic acid demonstrate more potent EC_{50 TA} values towards the germinant and are still inhibited by CDCA, possibly explaining the previous observations. By comparing the germination kinetics and the abundance of proteins in the germinant receptor complex, we revise our original model for CspC-mediated activation of spore germination and propose that CspC may activate spore germination and then inhibit downstream processes.

CLATHRIN-INDEPENDENT ENDOCYTOSIS OF CLOSTRIDIUM DIFFICILE TOXIN A

Chandrasekaran, R.;*1 Lacy, D.B.^{1,2}

¹Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN USA

²The Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN USA

Clostridium difficile infection (CDI) affects a significant number of hospitalized patients in the United States. Two homologous exotoxins, TcdA and TcdB, are the major virulence factors in *C. difficile* pathogenesis. The toxins are glucosyltransferases that inactivate Rho family-GTPases to disrupt host cellular function and cause fluid secretion, inflammation, and cell death. Toxicity depends on receptor binding and subsequent endocytosis. TcdB has been shown to utilize clathrin-dependent endocytosis to mediate Rac1 inactivation and cell rounding. However, investigations on TcdA uptake implicate both clathrin- and dynamin-dependent and independent pathways, and have relied primarily on inhibitor studies. Here, we utilize a combination of RNAi-based knockdown, pharmacological inhibition and cell imaging approaches to investigate the endocytic mechanism(s) that contribute to TcdA uptake, and subsequent toxin-induced Rac1 inactivation and cell death. We show that TcdA uptake and cellular intoxication is dynamin-dependent but does not involve clathrin- or caveolae-mediated endocytosis. Confocal microscopy using fluorescently labeled TcdA show that the toxin is internalized into pacsin2-positive membrane tubules that can undergo dynamin-dependent scission and release into the cytosol. Disruption of pacsin2 function by RNAi-based knockdown approaches inhibits TcdA uptake and toxin-induced cell death in Caco-2 cells. We conclude that TcdA and TcdB utilize distinct endocytic mechanisms to intoxicate epithelial cells.

SXII-3 SXII-4

CDTR – [ONLY?] THE REGULATOR OF BINARY TOXIN IN CLOSTRIDIUM DIFFICILE

Bilverstone, T.;¹ Kinsmore, N.L.;¹.² Stevenson, E.;¹.² Minton, N.P.;¹.² Kuehne, S.A.*¹.²

¹Clostridia Research Group, BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), The University of Nottingham, UK

²NIHR Nottingham Digestive Diseases Biomedical Research Unit at Nottingham University Hospitals NHS Trust and The University of Nottingham, UK

Clostridium difficile is a Gram-positive, endospore forming, anaerobic bacterium, responsible for most hospital acquired antibiotic induced diarrhoeal disease. The spores act as mean of transmission, while the disease itself is toxin mediated. Most strains produce two large glycosylating toxins, named toxin A and B, but about 20% of clinical isolates produce an additional binary toxin, called *C. difficile* toxin (CDT). CDT has been implicated in more severe infections and also in relapse, but little is known about its actual contribution to pathogenesis and disease outcome. Some evidence has been obtained that CDT might play a role in colonisation and there is also further data suggesting that it might act in concert with the large toxins. It is thought to be regulated by a LytTR response regulator, CdtR, encoded just upstream. Interestingly most strains that do not carry functional CDT genes have a ghost locus, encoding an intact copy of *cdtR*, but truncated *cdt* genes. Very few strains do not have *cdtR*; instead, these carry a conserved 68bp fragment.

Here we are exploring the following two research questions: Is CdtR absolutely required for functional binary toxin? Has CdtR further functions (in addition to binary toxin regulation)?

To address these, clean deletion mutants of *cdtR* have been generated in a ribotype 027 *C. difficile* strain (R20291, producing the large toxins A and B and also CDT) and also in CD630, containing the afore mentioned ghost locus. Furthermore the PaLoc (containing toxin A and B) has been deleted from R20291. Production of CDT is assessed in the R20291 mutants by Western blot and cytotoxicity. Effects of the CdtR deletion in a CDT negative strain are being investigated to potentially elucidate a role beyond binary toxin regulation.

The here presented study will give an insight into the role(s) and regulation of the binary toxin regulator CdtR in *C. difficile*.

LEPTIN SIGNALING ALTERS NEUTROPHIL HOMEOSTASIS DURING CLOSTRIDIUM DIFFICILE INFECTION

Madan, R.;*1 Xue, J.;1 Petri Jr., W.A.;2 Deepe Jr., G.1

¹Division of Infectious Diseases, University of Cincinnati, Cincinnati, OH USA

²Division of Infectious Diseases, University of Virginia, Charlottesville, VA USA

Background: *Clostridium difficile* is the leading cause of nosocomial infections in the US. Neutrophils play a central role in pathogenesis of *C. difficile*-associated disease: higher systemic neutrophilia (peak peripheral white cell count $> 20 \times 10^9$ cells/L) is associated with increased risk of mortality, and an over-exuberant neutrophil response in tissues contributes to colonic pathology. Leptin is an adipocyte-secreted cytokine that has diverse biological functions: regulation of food uptake, energy expenditure and inflammation, thus linking nutrition and immunity. We have previously demonstrated an important role for leptin signaling in *C. difficile* disease: European-Americans homozygous for the derived/mutant G allele of LEPR (RR genotype) had increased risk for *C. difficile* infection (OR 3.03, p = 0.015).

Methods and Results: In the current studies, we investigated the role of leptin signaling in controlling peripheral and tissue neutrophilia after *C. difficile* challenge in a mouse model. In mice with the same genetic mutation as humans (RR genotype), we show that 3 days after *C. difficile* infection, <u>RR mice have decreased systemic neutrophils</u> as compared to wildtype mice. RR mice also have <u>lower numbers of tissue neutrophils</u> 6 hours after intra-cecal challenge with *C. difficile* toxin A. RR genotype leads to diminished STAT3 signaling after leptin challenge. Congruent with our results in RR mice, mice with <u>uncoupled LEPR-STAT3</u> signaling had fewer cecal neutrophils, and less tissue inflammation (cecal histology) 2 days after *C. difficile* infection.

Conclusion: Our studies have uncovered an important pathway that connects metabolism with inflammation, and augments systemic and local neutrophilia in response to infectious challenge. We show that signaling from LEPR wildtype allele (and LEPR→STAT3 axis) produces a more vigorous neutrophil response. We are now studying the impact of leptin signaling on levels of cytokines that influence neutrophil production, egress from bone marrow and extravasation into infected tissues in mouse models of *C. difficile* infection.

Thursday, July 14, 2016 Clostridium difficile Management

INTRASPECIFIC COMPETITION AND ADAPTIVE IMMUNE
RESPONSES IN PROTECTION AGAINST MURINE
CLOSTRIDIUM DIFFICILE INFECTION

Leslie, J.L.;¹ Jenior, M.L.;¹ Young, V.B.*^{1,2}
¹Department of Microbiology & Immunology
²Department of Internal Medicine/Infectious Diseases Division, University of Michigan Medical School, Ann Arbor, MI USA

Purpose: To compare the relative contributions of adaptive immunity and intraspecific bacterial competition in protection against *C. difficile* infection.

Introduction: Naturally occurring and monoclonal anti-toxin antibodies have been shown to protect from *C. difficile* infection (CDI) in patients and animal models of disease. Additional work has demonstrated that intraspecific competition via treatment with a non-toxigenic *C. difficile* strain can also protect from infection with toxigenic strains. We sought to determine the relative contribution of intraspecific bacterial competition versus development of humoral antitoxin antibody response in providing protection from re-infection with *Clostridium difficile*.

Methods: We utilized two well-characterized lab strains (630 and VPI 10463) that are differentially virulent in our mouse model of CDI, yet express nearly identical forms of the toxins TcdA and TcdB. We used our murine model of CDI to perform sequential challenge with these two strains in wild type and RAG-/- animals.

Results: In wild type mice, prior infection with the less virulent strain 630 would protect against a normally lethal challenge with VPI 10463. This was associated with the development of anti-toxin antibodies. In RAG-deficient animals, we still observed protection, but only if strain 630 persisted. RAG-/- animals that spontaneously cleared 630 were susceptible to lethal VPI 10463 challenge. Metabolic modeling suggests that intraspecific competition between the two *C. difficile* strains depended on the ability to carbohydrates within the intestinal lumen.

Conclusions: This is the first study to evaluate the relative contributions of two distinct mechanisms proposed for protection from CDI. These results suggest that current efforts to develop novel therapies based on anti-toxin antibodies/vaccines and the use of competitive *C. difficile* strains both have a mechanistic basis for their activity. Therefore, multifaceted efforts to develop novel therapies for CDI hold promise for increasing the available armamentarium against *C. difficile*.

845	SESSION XIII: EVOLVING MANAGEMENT OF CLOSTRIDIUM DIFFICILE INFECTION	
SXIII-1	Antibiotics: New and in Clinical Research Trials <i>Johnson, S.</i> *	66
SXIII-2	Vaccines to Prevent Clostridium difficle Infections Ghose-Paul, C.*	67
SXIII-3	Biotherapeutics and Immunologics for CDI Prevention Gerding, D.N.*	68
SXIII-4	Safety and Efficay of RBX2660 for Recurrent <i>Clostridium difficile</i> Infection: Results of Phase 2 Punch CD Studies <i>Dubberke, E.;* Lee, C.H.; Orenstein, R.; Khanna, S.</i>	69

ANTIBIOTICS: NEW AND IN CLINICAL RESEARCH TRIALS

Johnson, S.*^{1,2}

¹Hines VA Hospital, Hinmes, IL USA

²Loyola University Medical Center, Chicago, IL USA

Development and approval of fidaxomicin in 2011 was a milestone in therapeutics for *C. difficile* infection (CDI). This macrocyclic, non-absorbed antibiotic that inhibits RNA polymerase was the first FDA-approved drug for CDI since the approval of vancomycin 31 years earlier. Fidaxomicin was shown in two large, multi-center, randomized, controlled, phase 3 trials to be non-inferior to vancomycin for cure (resolution of symptoms after 10 days) and superior for sustained clinical response (SCR) 1 month after treatment. SCR was also higher for fidaxomicin in a subgroup of patients treated for a first CDI recurrence. Antibiotics for CDI will likely remain the cornerstone of treatment in the future given the ubiquitous sources of exposure to the organism, inability to avoid risk factors for host intestinal dysbiosis (e.g., systemic antibiotics), and the fact that most of the non-antibiotic therapeutics in development are being studied as adjunctive treatments during or immediately after antibiotic treatment for CDI.

Several new antibiotics for CDI have completed pre-clinical, phase 1, and phase 2 clinical trials. All of these agents have minimal systemic absorption, are highly active against *C. difficile* in vitro, but have differing spectrums of activity against other intestinal commensal bacteria. LFF57, a thiopeptide antibiotic that inhibits prokaryotic translation, appeared to be similar in efficacy to vancomycin for cure and SCR in a phase 2 trial, and this drug has not undergone phase 3 testing. Preliminary results of a phase 2 clinical trial of ridinilazole, were recently reported. Based on encouraging SCR rates (66.7% versus 42.4% for vancomycin), this novel small molecule antibiotic with unknown mechanism of action, is being evaluated for phase 3 testing. Cadazolid, a novel fluoroquinolone-oxazolidinone antibiotic that acts by inhibiting bacterial protein synthesis is currently undergoing phase 3 testing. Phase 2 testing showed numerically higher SCR rates with cadazolid at 3 different dosages (46.7–60.0%) compared with vancomycin (33.3%). Finally, preliminary results have been reported for the first phase 3 trial of surotomycin, a lipopeptide structurally similar to daptomycin. Despite encouraging phase 2 results, the non-inferiority endpoint was not met for cure (79 % vs. 83.6%) or SCR (60.6% vs. 61.4 %). Further clinical trials will be needed to determine if ridinilazole, cadazolid, or other drugs in early development will become available in the future for treatment of CDI.

66

VACCINES TO PREVENT CLOSTRIDIUM DIFFICLE INFECTIONS

Ghose-Paul, C.* Symbiotic Health Inc., New York, NY USA

The incidence and severity of *Clostridium difficile* infection (CDI) have risen dramatically in the 21st century, leading to increased CDI-related morbidity and mortality. The pathogenicity of *C. difficile* is primarily mediated through toxin-mediated damage to the intestinal epithelium. Clinical evidence suggests that antibody responses to these toxins appear to be protective against primary disease and against recurrence. Given the importance of the host immune response against symptomatic disease, an effective *C. difficile* vaccine could be considered for both prophylactic and therapeutic applications. Over the past decade, a variety of toxin-based vaccines have been tested in preclinical animal models and clinical trials. In addition to toxin-specific vaccines, vaccines targeting nontoxin antigens may be needed to prevent colonization, reduce spore production, and interrupt disease transmission.

67

BIOTHERAPEUTICS AND IMMUNOLOGICS FOR CDI PREVENTION

Gerding, D.N.*^{1,2}
¹Loyola University Chicago, Chicago, IL USA
²Hines VA Hospital, Hines, IL USA

Biotherapeutics (live bacterial organisms other than FMT and its derviatives) and Immunologics (monoclonal antibodies and vaccines) have completed clinical trials for the prevention of recurrent CDI. This presentation will not include discussion of traditional probiotics which have been the subject of meta-analyses or vaccines that are the subject of another presentation.

The primary immunologics that have completed two phase 3 randomized, prospective, blinded trials, MODIFY I and MODIFY II, are the monoclonal antibodies actoxumab (acto) and bezlotoxumab (bezlo), directed against toxin A and toxin B of C. difficile respectively. Both were administered intravenously to >1800 patients while they underwent standard of care (SOC) treatment of CDI (first episode, or any recurrent CDI) with metronidazole, vancomycin, or fidaxomicin. Each was tested as a single agent and in combination. The primary endpoint was CDI recurrence with Global Cure as a secondary endpoint. An interim analysis of MODIFY I for acto alone indicated no benefit over placebo and it was dropped from MODIFY I and not included in MODIFY II. The combination of both monoclonals was not statistically different from bezlo alone, and the final analysis was done for bezlo vs placebo. Combined MODIFY I and II data showed bezlo CDI recurrence was 17% compared to 27% for placebo (p<.0001). An increase in Global Cure from 54% placebo to 64% bezlo (p<.0001) was also found. Patients at high risk for recurrence had similar or greater reductions with bezlo compared to placebo.

Non-toxigenic C. difficile strain M3 (NTCD-M3) is a naturally occurring strain of *C. difficile* that lacks the genes for production of toxins A, B, and binary toxin. NTCD-M3 was found safe in human volunteers at doses as high as 108 spores a day for 14 days. A phase 2 randomized, prospective, double-blind trial of spores of NTCD-M3 was conducted in 173 patients with first episode or first recurrence of CDI. Patients were treated with SOC (metronidazole, vancomycin, or both) to resolution of symptoms, then randomized to receive oral liquid formulation of NTCD-M3, 10⁴ spores/d for 7 days (n = 43), 10^7 spores/d for 7 days (n = 44), or 10^7 spores/d for 14 days (n = 42), or placebo for 14 days (n = 44) beginning the day after the last dose of antibiotic. Results: Fecal colonization occurred in 69% of NTCD-M3 patients: 71% with 10⁷ spores/d and 63% with 10⁴ spores/d. Recurrence of CDI occurred in 13 (30%) of 43 placebo patients and 14 (11%) of 125 NTCD-M3 patients (P = .006); the lowest recurrence was in 2 (5%) of 43 patients receiving 10^7 spores/d for 7 days (P = .01 vs placebo]). Recurrence occurred in 2 (2%) of 86 patients who were colonized vs 12 (31%) of 39 patients who received NTCD-M3 and were not colonized (P < .001).

Bezlotoxumab and NTCD-M3 show high promise to prevent recurrent CDI.

SAFETY AND EFFICAY OF RBX2660 FOR RECURRENT CLOSTRIDIUM DIFFICILE INFECTION: RESULTS OF PHASE 2 PUNCH CD STUDIES

Dubberke, E.;*1 Lee, C.H.;² Orenstein, R.;³ Khanna, S.⁴
¹Washington University School of Medicine, St. Louis, MO USA
²McMaster University, Hamilton, ON, Canada
³Mayo Clinic, Arizona, Phoenix, AZ USA
⁴Mayo Clinic, Rochester, MN USA

Background: *Clostridium difficile* infection (CDI) is a large public health concern with high morbidity, mortality and costs. Recurrent infection is challenging to treat. Disruption of the gut microbiota often via antimicrobial exposure is a risk factor for CDI. A standardized microbiota-based drug may enable a more accessible therapeutic option.

Objective: Assess the safety and efficacy of RBX2660, a commercially prepared microbiota-based drug, for the treatment of recurrent CDI.

Methods: Patients with recurrent CDI, defined as at least 3 CDI episodes or at least 2 severe episodes resulting in hospitalization, were enrolled in the PUNCH CD and PUNCH CD 2 studies. PUNCH CD was a prospective, multicenter, open-label study; PUNCH CD 2 was a randomized, doubleblind, placebo-controlled study. Sourced from human-derived microbes and manufactured using standardized, quality controlled processes, RBX2660 is supplied in an enema format. Efficacy was defined as the absence of CDI though 56 days after the last dose of RBX2660. Adverse events (AEs) were rigorously solicited and systematically recorded throughout the studies.

Results: RBX2660 was 87% effective in preventing recurrent CDI at 56 days in the PUNCH CD study. A total of 188 AEs were reported in 28 patients, most commonly gastrointestinal, all of which were self-limited. Seven patients experienced 20 serious AEs, none of which were determined to be related to RBX2660 or its administration. Results of the randomized controlled PUNCH CD 2 study, which completed enrollment in December 2015, will enable a more nuanced understanding of this promising therapy, including the potential impact of patient selection (diagnosis, comorbidities) and dosing on safety and efficacy.

Conclusions: RBX2660, a commercially prepared Microbiota Restoration Therapy, was well-tolerated, with 87% efficacy for preventing recurrent CDI at 56 days. The PUNCH CD 2 trial will provide evidence from the largest randomized controlled trial to date of a microbiota-based therapy for recurrent CDI.



Thursday, July 14, 2016 Clostridium Difficile in Europe SESSION XIV: CLINICAL EXPERIENCE OF CLOSTRIDIUM 1025 **DIFFICILE IN EUROPE** SXIV-1 Clinical Experience of Clostridium difficile in Europe: Introduction; Historical Perspectives 72 Nord, C.E.* SXIV-2 Diagnosis and Epidemiology of CDI in Europe 73 Kuijper, E.J.* SXIV-3 Treatment of Primary and Recurrent CDI in Europe 74

Wilcox, M.H.*

SXIV-1 SXIV-2

CLINICAL EXPERIENCE OF CLOSTRIDIUM DIFFICILE IN EUROPE: INTRODUCTION; HISTORICAL PERSPECTIVES

Nord, C.E.*

Department of Laboratory Medicine, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden

Clostridium Difficile (Bacillus difficilis) was first described in the intestinal microflora of newborn infants by Hall and O'Toole 1935. In 1978, Larson and co-workers reported the association between pseudomembranous colitis and C. difficile infection. Epidemiological data on C. difficile infections from Sweden were reported 1980-1982. Aronson, et. al. 1982, characterized toxin A from C. difficile in patients with antibiotic associated colitis. The use of immunological methods for testing antibodies to C. difficile toxins in patients were published 1983. Tvede (1989) gave the first report about the successful use of characterized bacteria in faecal microbiota transplantation in six patients with recurrent C. difficile Infections. Ribotyping of C. difficile with 116 different PCR types was introduced by Brazier et al 1999 in Cardiff, Wales.

During the last ten years the interest of *C. difficile* infections in Europe has significantly increased. Many papers have been published in international scientific journals reporting new laboratory and clinical data on *C. difficile*. The most active collegues in Europe have been and are Kuijper's and Wilcox's groups. Different European organisations working with *C. difficile* infections are described below.

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) study group on *Clostridium difficile* (ESGCD) is very active in arranging European symposia, workshops, and postgraduate education courses. The study group has also published several guidelines for diagnosis and treatment of *C.difficile* infections.

The European Centre for Disease Prevention and Control (ECDC) also has a program for *C.difficile* infections in Europe.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) published a paper in 2012 describing antimicrobial susceptibility testing of *C. difficile* using the EUCAST disk diffusion method.

CDI Europe is a working group of experts interested in *C. difficile* infections in Europe. The group is focused on diagnosis, treatment, prevention, infection control, surveillance, patient empowerment, patient safety, and care quality in the European Union.

Clostridium difficile infection is now the leading cause of health-care associated infections in Europe.

DIAGNOSIS AND EPIDEMIOLOGY OF CDI IN EUROPE

Kuijper, E.J.*

Department Medical Microbiology and Experimental Bacteriology, Leiden University Medical Centre, Leiden, The Netherlands

Clostridium difficile infection (CDI) remains poorly controlled in many European countries, of which several have not yet implemented national CDI surveillance. Suboptimal laboratory diagnostics for CDI impedes its surveillance and control across Europe. In 2010, the ECDC launched a new project, i.e. the European *C. difficile* Infection Surveillance Network (ECDIS-Net), to enhance surveillance and laboratory capacity for CDI in Europe. We evaluated changes in local laboratory CDI diagnostics and changes in national diagnostic and typing capacity for CDI during 'ECDIS-Net' through cross-sectional surveys in 33 European countries in 2011 and 2014. Amongst laboratories that participated in both surveys, use of CDI diagnostics deemed 'optimal' or 'acceptable' increased from 19% to 46% and from 10% to 15%, respectively (p = <0.001). Subsequently, an update of the guidance document for CDI diagnostics was made and approved by the European Society for Clinical Microbiology and Infectious Diseases (in press). In 2013, a protocol was developed with three options of CDI surveillance for acute care hospitals: a 'minimal' module option (aggregated hospital data), a 'light' module option (including patient data for CDI cases) and an 'enhanced' surveillance module option (including microbiological data on the first 10 CDI episodes). Thirty-seven hospitals in 14 European countries tested these options for a three-month period. A total of 1,152 CDI episodes were recorded by 'minimal' surveillance. After exclusion of recurrent episodes, the incidence of healthcare-associated CDI by hospital ranged from 4.2 to 131.8 per 10,000 discharges (median: 16.4; IQR: 10.1-29.5) and from 0.6 to 18.5 per 10,000 patient-days (median: 3.7; IQR: 2.0-6.6). The current hospital was reported as being the origin of infection for 66% (n = 673), another hospital for 18% (n = 178), a long-term care facility for 1% (n = 13) and another healthcare facility for 2% (n = 21) of the episodes. Fifty-one different PCR ribotypes were characterised. The pre-dominant PCR ribotype was 027 (30%), followed by the highly related PCR ribotypes 014 and 020 (15%), and PCR ribotype 001 (6%). PCR ribotype 027 was identified in isolates from eight (62%) European countries in 4%-85% of all characterised samples depending on the country. We conclude that standardised multicountry surveillance with the option of integrating clinical and molecular data is a feasible strategy for monitoring CDI in Europe.

Thursday, July 14, 2016

Clostridium difficile Pathogenesis

TREATMENT OF PRIMARY AND RECURRENT CDI IN EUROPE

Wilcox, M.H.*
University of Leeds, Leeds Teaching Hospitals & Public Health, Leeds, England UK

UK (Public Health England) and ESCMID guidelines for the treatment of CDI were published in 2013 and 2014, respectively. There are broad similarities, but the important differences between these guidelines will be discussed here. Notably, both guidelines were published before recently published evidence casts significant doubt on the utility of metronidazole as a mainstay treatment for CDI. We, therefore, have a gap between current practice and supporting evidence, as metronidazole remains commonly prescribed for CDI in some, but not all, countries in Europe.

The position of and stated level of evidence for fidaxomicin in guidelines varies. Phase 3 studies likely reflecting high acquisition cost. A 2012 UK NICE Evidence Summary states that 'When considering the use of fidaxomicin, local decision makers should take into account the potential benefits alongside the medical need, the risks of treatment, and the relatively high cost of fidaxomicin in comparison with other treatments for CDI.' Interestingly, a recent UK study reported that fidaxomicin is cost-effective in patients with severe CDI and in patients with a first CDI recurrence versus vancomycin.

Another area of controversy is the positioning of faecal microbiota transplantation (FMT) in the treatment pathway for recurrent CDI. While FMT offers clear potential option for patients with multiple recurrences of CDI, there are unanswered questions regarding the route of administration, the necessary extent of screening of donors and recipients, the faecal transplant volume, and particularly the long term safety of gut microbiome manipulation.

Future possible treatment options for CDI include bezlotoxumab, a human monoclonal anti-toxin B antibody. This may represent a useful alternative for patients with and/or at high risk of recurrent CDI. In this context, recent data indicate that the cost and consequences of recurrence CDI are considerable.

1420	SESSION XV: AN UPDATE ON CLOSTRIDIUM DIFFICILE PATHOGENESIS	
SXV-1	Quorum Sensing in Clostridium difficile Darkoh, C.*	76
SXV-2	Adherence Mechanisms of <i>Clostridium difficile</i> to Gut Epithelial Cells	77
	Vedantam, G.*	
SXV-3	Unique Contributions of TcdA and TcdB to <i>Clostridium</i> difficile Disease Lyras, D.*	78
SXV-4	The Impact of Nutrition on <i>Clostridium difficile</i> Infection <i>Zackular</i> , <i>I.P.</i> *	79

QUORUM SENSING IN CLOSTRIDIUM DIFFICILE

Darkoh, C.*1,2

¹The University of Texas Health Science Center, School of Public Health, Department of Epidemiology, Human Genetics, and Environmental Sciences, Center for Infectious Diseases, Houston, TX USA ²The University of Texas Graduate School of Biomedical Sciences, Houston, TX USA

Clostridium difficile infection (CDI) is drastically increasing as a cause of antibiotic- and hospital-associated diarrhea worldwide. C. difficile, a Gram-positive multidrug-resistant anaerobic pathogen, overpopulates the colon after the gut microbiota has been altered by antibiotic therapy. Consequently, it produces toxins A and B that directly cause disease. Despite the enormous public health problem posed by this pathogen, the molecular mechanisms that regulate production of the toxins, which are directly responsible for disease, remained largely unknown until now. Using an unbiased biochemical and genetic approach and a classic quorum signaling bioassay, we have established that *C. difficile* toxin synthesis is regulated by quorum signaling through a novel autoinducing peptide (*CdAIP*). This novel peptide is encoded by a homolog of the Gram-positive specific accessory genes regulator (agr) quorum signaling system. The purified CdAIP induced early transcription of the C. difficile toxin genes and stimulated elevated toxin production in all clinical isolates. Furthermore, the CdAIP was detected only in stools from CDI-positive patients, but not in CDI-negative stools. Interestingly, some strains of *C. difficile* possess two loci of the agr-component genes, designated agr1 and agr2 loci. The agr1 locus is present in all of the strains sequenced to date, including the historical 630 strain, and contains only the quorum signaling autoinducing peptide generation genes (agrB1 and D1). On the other hand, the agr2 locus, which is present mostly in few strains including the hypervirulent NAP1/027 R20291 strain, contains both autoinducing peptide generation and response genes (agrB2D2 and agrC2A2, respectively). Using allelic exchange, we deleted both agrB1D1 and agrB2D2 loci in the R20291 strain and examined the mutants for toxin production and virulence. The results showed that both *C*. difficile strains R20291 and 630 agrB1D1 mutants cannot produce the A and B toxins and toxin production can be restored by complementation with wildtype agrB1D1 gene. Furthermore, both R20291 and 630 agrB1D1 mutants are unable to cause disease in a murine infection model. These findings underscore the clinical relevance of the agr system in *C. difficile* pathogenesis and opens up unique therapeutic targets for the development of a nonantibiotic anti-quorum sensing therapy for CDI.

ADHERENCE MECHANISMS OF *CLOSTRIDIUM DIFFICILE* TO GUT EPITHELIAL CELLS

Vedantam, G.* University of Arizona, Tucson, AZ USA

Clostridium difficile is a Gram-positive, anaerobic, spore-forming, enteric pathogen. It is a causative agent of antibiotic-associated diarrhea and, in a subset of patients, can engender severe sequelae. *C. difficile* infection (CDI) impacts healthcare systems across North America; over 500,000 CDI cases occur annually in the U.S.A. alone.

Toxins A and B (TcdA and TcdB respectively), the primary *C. difficile* virulence factors, are significant mediators of intestinal damage and pathology. However, the contributions of non-toxin virulence factors to *C. difficile* colonization and disease are beginning to be increasingly appreciated. Like many enteric pathogens, *Clostridium difficile* must associate with the intestinal mucosa to begin the process of host colonization. Multiple *C. difficile* adhesins have been described, including the flagellin FliC, the flagellar cap protein FliD, fibronectin-binding proteins, a heat-shock protein GroEL, the surface associated, heat-shock-induced adhesin, Cwp66, and the surface layer protein, SlpA. SlpA contains two biologically distinct entities, the high-molecular weight (HMW) and the low molecular weight (LMW) subunits, which are derived via cleavage of a precursor protein, and which assemble on the bacterial surface into a paracrystalline lattice. The two subunits associate with high affinity through the N-terminus of the HMW protein and the C-terminus of the LMW protein.

SlpA is a major contributor to *C. difficile* adherence, and individual subunits of the protein (varying in sequence between strains) mediate host-cell attachment to different extents. Pre-treatment of host cells with crude or purified SlpA subunits, or incubation of vegetative bacteria with anti-SlpA antisera significantly reduces *C. difficile* attachment. SlpA-mediated adherence-interference correlates with the attachment efficiency of the strain from which the protein is derived, with maximal blockage observed when SlpA is derived from highly adherent strains. In addition, SlpA-containing preparations from a non-toxigenic strain effectively block adherence of a phylogenetically distant, epidemic-associated strain, and vice-versa. Taken together, these results suggest that SlpA is one factor that plays a major role in *C. difficile* colonization of the mammalian gastrointestinal tract.

UNIQUE CONTRIBUTIONS OF TCDA AND TCDB TO CLOSTRIDIUM DIFFICILE DISEASE

Lyras, D.*
Department of Microbiology, Monash University, Victoria, Australia

Most disease-causing strains of *Clostridium difficile* secrete two exotoxins, TcdA and TcdB. We have used three different animal models to gain a better understanding of how these toxins function during infection and have shown that TcdA+TcdB- mutants of a ribotype 027 strain, are attenuated in virulence in comparison to the wild type (TcdA+TcdB+) strain whereas TcdA-TcdB+ mutants are fully virulent. We also showed that TcdB alone is associated with both severe localized intestinal damage as well as systemic organ damage. Our results provide new insights into the host response to C. difficile during infection and show that TcdB is the primary factor responsible for inducing the *in vivo* host innate immune and inflammatory responses, with increased levels of numerous pro-inflammatory cytokines and chemokines found in colonic tissue isolated from mice infected with the TcdA-TcdB+ mutants, at levels similar to those detected in tissues from mice infected with the wild-type strain. Cytokine induction coincided with the development of severe colonic damage and fulminant disease. Surprisingly, the animal infection model used was found to profoundly influence disease outcomes, a finding which has important ramifications for the validation of new therapeutics and which highlights the critical nature of using appropriate and, when possible, multiple animal infection models when studying bacterial virulence mechanisms. Overall, this work provides valuable insights into the unique contributions of TcdA and TcdB to *C. difficile* disease and the host immune response.

THE IMPACT OF NUTRITION ON CLOSTRIDIUM DIFFICILE INFECTION

Zackular, J.P.* Vanderbilt University, Nashville, TN USA

Clostridium difficile is the most commonly reported nosocomial pathogen in the United States and is an urgent public health concern worldwide. Over the past decade, incidence, severity, and costs associated with *C*. difficile infection (CDI) have increased dramatically. CDI is most commonly initiated by antibiotic-mediated disruption of the gut microbiota; however, non-antibiotic associated CDI cases are well documented and on the rise. This suggests that unexplored environmental, nutrient, and host factors likely influence CDI. Here we show that increased levels of dietary zinc (Zn) alter the structure and diversity of the gut microbiota. These Zn-mediated community alterations considerably reduce the threshold of antibiotics needed to eliminate colonization resistance to *C. difficile*. Moreover, in mice colonized with C. difficile, excess dietary Zn exacerbates C. difficileassociated disease by increasing C. difficile toxin production and altering the host immune response. In addition, we have determined that the host metal binding protein calprotectin is essential for combating C. difficile by limiting Zn at the site of infection. Together, these data demonstrate that nutrient Zn levels play a key role in determining susceptibility to CDI and severity of C. difficile-associated disease. These discoveries will lay the groundwork for the development of novel preventative and therapeutics strategies for CDI.



Thursday, July 14, 2016

Clostridium difficile II

1615	SESSION XVI: ORAL ABSTRACTS: CLOSTRIDIUM DIFFICILE II	
SXVI-1	Acute Gastroenteritis in Children Using Multiplex Nucleic Acid-Based Testing and the Role of Clostridium difficile Nicholson, M.R.;* Van Horn, G.T.; Tang, Y.W.; Little, J.L.; Zhu, Y.;	82
	Vinjé, J.; Payne, D.C.; Edwards, K.M.; Chappell, J.D.	
SXVI-2	Oral Immunization with Non-Toxic <i>C. difficile</i> Strains Expressing Chimeric Fragments of TcdA and TcdB Elicit Protective Immunity Against <i>C. difficile</i> Infection in Both Mice and Hamsters	83
	Wang, Y.;* Bouillaut, L.; Ju, X.; Wang, Y.; Sonenshein, A.L.; Sun, X.	
SXVI-3	Clostridium difficile Infection in South-East Asia	84
	Collins, D.A.; Putsathit, P.; Gasem, M.H.; Habibie, T.; Arinton, I.G.; Handrianto, P.; Agung, I.G.; Lee, Y.Y.; Hassan, S.A.; Nadiah, H.Z.; Kiratisin, P.; Elliott, B.; Riley, T.V.*	
SXVI-4	Disease Progression and Resolution in Rodent Models of <i>Clostridium difficile</i> Infection: Impact of Antitoxin Antibodies	85
	Warn, P.; Thommes, P.; Sattar, A.; Flattery, A.; Zhang, Z.; Hernandez, L.D.; Therien, A.G.*	
SXVI-5	Dissecting the Assembly Mechanism and Functional Role of the Outermost Exosporium Layer of <i>Clostridium difficile</i> Spores	86
	Calderón-Romero, P.; Olguín-Araneda, V.; Milano-Céspedes, M.; Plaza-Garrido, Á.: Sarker, M.R.: Paredes-Sahia, D.*	

SXVI-1 SXVI-2

ACUTE GASTROENTERITIS IN CHILDREN USING MULTIPLEX NUCLEIC ACID-BASED TESTING AND THE ROLE OF *CLOSTRIDIUM DIFFICILE*

Nicholson, M.R.;*1 Van Horn, G.T.;² Tang, Y.W.;³ Little, J.L.;⁴ Zhu, Y.;⁵ Vinjé, J.;⁶ Payne, D.C.;⁶ Edwards, K.M.;⁷ Chappell, J.D.⁴

¹Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Vanderbilt University School of Medicine, Nashville, TN USA

²Department of Microbiology, American Esoteric Laboratories

³Department of Laboratory Medicine, Memorial Sloan-Kettering Cancer Center, Weill Medical College of Cornell University, New York, NY USA

⁴Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN USA

⁵Department of Biostatistics, Vanderbilt University, Nashville, TN USA

⁶Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA USA

⁷ Vanderbilt Vaccine Research Program, Division of Pediatric Infectious Disease, Vanderbilt University School of Medicine, Nashville, TN USA

Purpose: To determine the etiologic profile of acute gastroenteritis in children using multiplex nucleic acid-based testing and compare clinical presentations between etiologies.

Methods: This was a prospective surveillance study of pediatric patients with acute gastroenteritis conducted from 2008-2011 as part of the New Vaccine Surveillance Network. Stools from patients and healthy controls were tested for a total of 21 gastrointestinal pathogens using the Luminex *Analyte Specific Reagent Gastrointestinal Pathogen Panel* (Luminex Molecular Diagnostics) and a reverse transcription real-time polymerase chain reaction assay. Children with a single viral pathogen, bacterial pathogen, no pathogen, or more than one pathogen identified were compared on multiple clinical variables.

Results: Of the 216 stool samples from patients with acute gastroenteritis, 152 (70%) tested positive for at least one pathogen, with norovirus GII (78, 36.1%) and *C. difficile* (35, 16.2%) being the most common detections. A pathogen was identified in 4/36 (11%) of asymptomatic controls; *C. difficile* in three and norovirus GII in the remaining one. Of the 35 patients positive for *C. difficile*, 25 (71%) were also positive for another pathogen. *C. difficile* was more prevalent in children <3 years of age, with 94% of *C. difficile* detections in these young children. Children who had a co-infection did not differ clinically from those with a single viral, single bacterial, or no pathogen identified when compared on multiple clinical parameters.

Conclusions: Using a broad molecular testing approach, high rates of enteropathogens were detected in children with acute gastroenteritis, dominated by norovirus GII and *C. difficile*. Co-infections were common with *C. difficile* and these children did not differ clinically from other children in the cohort. *C. difficile* was also not uncommonly detected in asymptomatic children. As routine diagnostics of pediatric acute gastroenteritis progressively evolve toward nucleic acid-based pathogen detection, the role of and necessity for treatment of *C. difficile*, particularly in young children needs to be further defined.

ORAL IMMUNIZATION WITH NON-TOXIC C. DIFFICILE STRAINS EXPRESSING CHIMERIC FRAGMENTS OF TCDA AND TCDB ELICIT PROTECTIVE IMMUNITY AGAINST C. DIFFICILE INFECTION IN BOTH MICE AND HAMSTERS

Wang, Y.;*1,3 Bouillaut, L.;² Ju, X.;¹ Wang, Y.;¹ Sonenshein, A.L.;² Sun, X.1,3

¹Department of Infectious Disease and Global Health, Tufts University Cummings School of Veterinary Medicine, North Grafton, MA USA ²Department of Molecular Biology and Microbiology, Tufts University School

of Medicine, Boston, MA USA

³Department of Molecular Medicine, Morsani School of Medicine, University of South Florida, Tampa, Fl USA

The symptoms of *Clostridium difficile* infection (CDI) are attributed largely to two C. difficile toxins, TcdA and TcdB. Significant efforts have been devoted to developing vaccines targeting both toxins through parenteral immunization routes. However, accumulating evidence shows that anti-TcdA IgG, but not IgA, dramatically enhances TcdA-mediated cytotoxicity in vitro and disease in vivo, raising safety concerns with parenteral immunization. In addition, C. difficile is an enteric pathogen, and mucosal/ oral immunization would be particularly useful to protect the host against CDI considering that the gut is the main site of disease onset and progression. Moreover, vaccines directed only against toxins do not target the cells and spores that transmit the disease. Previously, we constructed a chimeric vaccine candidate mTcd138, comprised of the glucosyltransferase and cysteine proteinase domains of TcdB and the receptor binding domain of TcdA. Purified mTcd138 induced protective immunity against *C.difficile* challenge in mice and hamsters. In the current study, we exressed mTcd138 in a non-toxigenic *C. difficile* strain with the goal of developing an oral vaccine that targets both *C. difficile* toxins and colonization/adhesion factors. mTcd138 expression in the non-toxigenic C. difficile strain was verified by Western-blot analysis. Oral immunization with spores of the non-toxigenic strain expressing mTcd138 provided mice with full protection against the clinically relevant C. difficile strain UK1 (ribotype 027). The protective strength and efficacy of the mTcd138-expressing non-toxigenic C. difficile strain were further tested in the acute hamster model of CDI. Oral immunization with spores of the mTcd138-expressing non-toxigenic strain also provided hamsters significant protection against infection with 10⁴ spores of UK1, a dose100-fold higher than the lethal dose of UK1 in hamsters. In conclusion, the genetically modified, non-toxigenic C. difficile strain expressing mTcd138 represents a novel mucosal vaccine candidate against CDI.

SXVI-3 SXVI-4

CLOSTRIDIUM DIFFICILE INFECTION IN SOUTH-EAST ASIA

Collins, D.A., Putsathit, P.; Gasem, M.H.; Habibie, T., Arinton, I.G., Handrianto, P.; Agung, I.G.; Lee, Y.Y.; Hassan, S.A.; Nadiah, H.Z., Kiratisin, P.; Elliott, B., Riley, T.V.

¹School of Pathology & Laboratory Medicine, University of Western Australia, Nedlands, Australia

²Dr. Kariadi Hospital, Diponegoro University, Semarang, Indonesia ³Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia ⁴Municipal Hospital, Semarang, Indonesia

⁵Kartini Hospital, Jepara, Indonesia

⁶School of Medical Sciences, Universiti Sains Malaysia, Kota Bahru, Malaysia ⁷Department of Microbiology, Faculty of Medicine, Mahidol University, Siriraj Hospital, Bangkok, Thailand

⁸Department of Microbiology, PathWest Laboratory Medicine, Nedlands, Australia

While *Clostridium difficile* infection (CDI) has come to prominence over the last 15 years because of major epidemics in North America and Europe, awareness and surveillance in Asia is poor. Limited studies suggest CDI may also be a significant problem in this region, but the true prevalence remains unknown particularly in S-E Asia. Poorly regulated antibiotic use in Asian countries could lead to high rates of CDI. We undertook studies of CDI in Indonesia, Malaysia, and Thailand. Faecal samples from patients with diarrhoea were investigated in the country of origin with either PCR for the tcdB gene or a lateral flow EIA to detect GDH/toxins. Samples were stored frozen and then transported to Western Australia for culture using Chrom ID agar and enrichment broth, followed by PCR for toxin genes and PCR ribotyping for molecular epidemiology. For samples from Indonesia (n=340), the overall prevalence of toxigenic *C. difficile* was 7.9%; for Malaysia (n=76), the prevalence was 9.2%; and for Thailand (n=422), the prevalence was 9.0%. Non-toxigenic strains were common, 12% to 15% prevalence. Binary toxin positive strains were not detected. Significant ribotype diversity was seen with most ribotypes having low single digit prevalence in all countries. However, ribotype 017 (A-B+) was highly prevalent: Thailand (11.4%), Malaysia (13.6%) and Indonesia (24.3%). These data suggest CDI is also a problem in S-E Asia, however, the molecular epidemiology is different to elsewhere. The significance of high rates of carriage of non-toxigenic strains needs investigation.

DISEASE PROGRESSION AND RESOLUTION IN RODENT MODELS OF *CLOSTRIDIUM DIFFICILE* INFECTION: IMPACT OF ANTITOXIN ANTIBODIES

Warn, P.;² Thommes, P.;² Sattar, A.;² Flattery, A.;¹ Zhang, Z.;¹ Hernandez, L.D.;¹ Therien, A.G.*¹ ¹Merck & Co., Inc, Kenilworth, NJ USA ²Evotec (UK) Ltd., Manchester UK

Clostridium difficile causes infections of the colon in patients with antibioticinduced gut dysbiosis. The symptoms of *C. difficile* infection (CDI) are caused by two exotoxins, TcdA and TcdB. The toxin-neutralizing antibodies actoxumab ("acto") and bezlotoxumab ("bezlo") are protective in animal models of CDI when administered in combination, and bezlo (with or without acto) reduces the rate of recurrent CDI in patients. In this study, mouse and hamster models of CDI were utilized to assess progression and resolution of CDI in animals treated with vehicle, acto+bezlo or vancomycin. Vehicle-treated animals challenged with C. difficile showed high intestinal levels of *C. difficile* within hours of challenge. Infection caused body weight loss, changes in body temperature, diarrhea, and severe intestinal pathology, and was fatal in 100% of hamsters and 75% of mice. Treatment with vancomycin delayed, but did not prevent, the onset of symptoms and death. Conversely, treatment with actoxumab and bezlotoxumab significantly reduced morbidity, intestinal damage and mortality, but not intestinal C. difficile burden. Animals that survived the acute phase of disease, regardless of treatment, showed complete resolution of symptoms and had largely undetectable levels of *C. difficile* bacteria in their intestines by Day 28 post-challenge. Intestinal microbiome composition, assessed in infected mice, was initially disrupted following the CDI-pre-conditioning antibiotic treatment received by all animals, but gradually reverted back to a "normal" composition, similar to naïve animals, by Day 28. In conclusion, rodent models of CDI are characterized by an acute phase of symptomatic disease, followed by gradual recovery of the gut microbiome associated with a clearance of *C. difficile* and resolution of disease symptoms over time. Treatment with antitoxin antibodies protects the host from toxin-dependent symptoms during the acute phase of disease, effectively preventing disease until the microbiome—the body's natural defense against *C. difficile*—has recovered.

84 85

DISSECTING THE ASSEMBLY MECHANISM AND FUNCTIONAL ROLE OF THE OUTERMOST EXOSPORIUM LAYER OF CLOSTRIDIUM DIFFICILE SPORES

Calderón-Romero, P.;¹ Olguín-Araneda, V.;¹ Milano-Céspedes, M.;¹ Plaza-Garrido, Á.;¹ Sarker, M.R.;².³ Paredes-Sabja, D.*¹.³

¹Gut Microbiota and Clostridia Research Group, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile

²Department of Microbiology, Oregon State University, Corvallis, OR USA ³Department of Biomedical Sciences, Oregon State University, Corvallis, OR USA

Clostridium difficile is a major cause of nosocomial antibiotic-associated diarrhea in developed and developing countries. Recurrent C. difficile infections are primarily due to the persistence of *C. difficile* spores in the host after antibiotic treatment. Evidence has demonstrated that C. difficile spores have an outermost layer, termed the exosporium, which is involved in host-spore interactions which seem to be relevant for the initiation and recurrence of the infection. However, the mechanism of exosporium assembly remains unclear. In this work, we have identified the protein composition of the exosporium layer and identified two cysteine rich exosporium proteins, CdeM and CdeC, which where characterized further. Through the construction of independent isogenic knockout mutants in the cdeC and cdeM genes of the C. difficile 630 strain their role in the exosporium assembly and function were evaluated. Transmission electron micrographs demonstrated that CdeC is required for the correct assembly of the exosporium layer, while CdeM is essential for the presence of this layer. Western blot analysis of fractions of spores expressing Flag-fusions of various exosporium proteins demonstrated the requirement of these morphogenetic factors in the presence of the relative abundance of these proteins in the exosporium fraction. Functional analysis of mutant spores demonstrated that *cdeC* and *cdeM* spores were more sensitive to lysozyme, ethanol, heat treatment, and macrophage infection than wild-type spores. In vivo colonization experiments suggest that while spores with an aberrantly assembled exosporium layer (cdeC spores) had a higher colonization index than wild-type, spores with absence of the exosporium layer (*cdeM* spores) exhibited a lower colonization index than wild-type spores. Collectively, these results shed light onto the mechanisms of assembly of the exosporium layer and the impact of this layer on functional properties of *C. difficile* spores.

1315	POSTER SESSION I: CLINICAL ABSTRACTS	
PI-1	Antibiotic Susceptibility of Anaerobes Isolated from Infected Wound Sites and Blood of Orthopedic Patients with Septicemia Ayepola, O.O.;* Egwari, L.O.; Nwokoye, N.N.; Olubi, O.O.; Faparusi, J.; Babalola, F.	89
PI-2	Septicaemia Following Orthorpedic Wound Infections	90
	Ayepola, O.O.;* Egwari, L.O.; Nwokoye, N.N.; Olubi, O.O.; Faparusi, F.	
PI-3	Prevalence and Antimicrobial Resistance of Anaerobic Bacteria in Infected Breasts of Turkish Women with Lactational Mastitis, Periductal and Granulomatous Mastitis Bahar-Tokman, H.;* Taner, Z.; Velidedeoglu, M.; Goksoy, E.; Celik, V.; Demirci, M.; Dal, F.; Kucuk, Y.; Mete, B.; Yemisen, M.;	91
DI 4	Kocazeybek, B.; Gunaydın, M.; Kiraz, N.	0.0
PI-4	Genomic Comparison of Multiple Campylobacter Rectus Strains Blackburn, D.;* Peeler-Fletcher, S.A.; Kinder, M.N.; Konganti, K.; Harrell, E.A.; Threadgill, D.S.	92
PI-5	Effect of Previous Beta-Lactam Therapy on Resistance to Beta- Lactam Antibiotics in Gram Negative Bacteria Isolated from Cases of Chronic Suppurative Otitis Media	93
	Egwari, L.O.;* Nwokoye, N.N.; Olubi, O.O.	
PI-6	Colorectal Cancer and Fusobacterium Nucleatum Infection	94
	Flynn, K.J.;* Schloss, P.D.	
PI-7	Antibiotic Susceptibility Profiles of Recent European Anaerobes: Test 2014-2015	95
	Hackel, M.;* Bailey-Person, M.; Sahm, D.; Leister-Tebbe, H.	
PI-8	Evaluation of Two Different Systems Installed on VITEK MS System for Identification of Clinically Isolated Anaerobic Bacteria Hayashi, M.;* Tanaka, K.	96
PI-9	Investigation of the Antibiotic Resistance Gene Content of Intestinal Normal Flora <i>Bacteroides</i> Strains Using a Novel Chromogenic Agar for the Isolation Sóki, J.; Jeverica, S.; Tierney, D.; Perry, J.D.; Nagy, E.;* Urbán, E.	97
PI-10	Detection and Preliminary Characterization of a Novel	
11-10	Mobilizable Transposon, MTnBf8, Harboring a Chromosomal nimB Gene	98
	Sóki, J.; Nagy, E.;* Urbán, E.	
PI-11	Epidemiology of Antibiotc Resistance of Clinically Relevant Bacteroides fragilis Group Isolates in Hungary Sarvari, P.K.; Nagy, E.;* Miszti, C.; Sóki, J.; Urbán, E.	99

Posters will be presented in Poster Session I Tuesday, July 12 1315-1415.

Clinical Abstracts

PI-12	Antimicrobial Susceptibility of <i>Propionibacterium acnes</i> Isolated from Ecuadorian Patients Who Were Under Anti-Microbial Therapy <i>Solís, M.B.;* Velasco, N.; Gonzalez, C.; Dressendorfer, L.M.; Zurita, J.</i>	100
PI-13	Antimicrobial Susceptibilities for 331 Strains of <i>Prevotella</i> Species Isolated in Japan	101
	Yamagishi, Y.;* Suematsu, H.; Nishiyama, N.; Koizumi, Y.; Mikamo, H.	
PI-14	Antimicrobial Susceptibilities for 67 Strains of <i>Peptostreptoccus</i> anaerobius Isolated in Japan	102
	Mikamo, H.; Yamagishi, Y.*; Suematsu, H.; Nishiyama, N.; Koizumi, Y.	
PI-15	The Role of Anaerobic Bacteria in Polymicrobial Necrotizing Wound Infections	103
	Zhao-Fleming, H.H.;* Rumbaugh, K.P.	
PI-16	The Role of Anaerobic Bacteria in Polymicrobial Infections Zhao-Fleming, H.H.:* Rumbaugh, K.P.	104

ANTIBIOTIC SUSCEPTIBILITY OF ANAEROBES ISOLATED FROM INFECTED WOUND SITES AND BLOOD OF ORTHOPEDIC PATIENTS WITH SEPTICEMIA

Ayepola, O.O.;*1 Egwari, L.O.;¹ Nwokoye, N.N.;² Olubi, O.O.;³ Faparusi, J.;¹ Babalola, F.¹

¹Department of Biological Sciences, College of Science and Technology, Covenant University, Canaanland, Ota, Ogun State, Nigeria ²National Tuberculosis Reference Laboratory, Microbiology Division, Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria ³Department of Ear, Nose and Throat, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

Anaerobic bacteria isolated from septic wound sites and positive anaerobic blood cultures of 12 orthopedic patients were evaluated for antibiotic susceptibility. Bacteroides fragilis was isolated from infected wounds of five patients of which two had positive B. fragils blood cultures. Finegoldia magna was isolated from seven infected wounds, but only three yielded F. magna positive blood culture. The Prevotella species were isolated from six infected wounds with two positive blood culture due to *P. intermedia*. The wound and blood isolates 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 in both wound and blood isolates were sensitive to the antibiotics except pencillin, and amoxicillin. One of the two *Bacteroides* species 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.

SEPTICAEMIA FOLLOWING ORTHORPEDIC WOUND INFECTIONS

Ayepola, O.O.;*1 Egwari, L.O.;¹ Nwokoye, N.N.;² Olubi, O.O.;³ Faparusi, F.¹ *¹Department of Biological Sciences, College of Science and Technology, Covenant University, Canaanland, Ota, Ogun State, Nigeria ²National Tuberculosis Reference Laboratory, Microbiology Division, Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria ³Department of Ear, Nose and Throat, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

Blood cultures for anaerobes are not routine practice in many hospitals in Nigeria, resulting in underreporting of the incidence of anaerobic septicemia in bloodstream infections. This also means absence of targeted treatment against anaerobes in septicemia requiring antibiotic intervention. Twelve patients that developed pyrexia following sepsis of orthopedic wounds were investigated. Four of the wounds were on the hip joints, six prosthetic joint infection (PJI), and two jaw fractures. Three of the patients with septic hip joints wounds were diabetic, and infections of the fractured jaw patients had persisted for 4 months (chronic infection). Both needle aspirates from infection sites and venous blood samples were processed for culture for aerobes and anaerobic bacteria. Coagulase negative staphylococci were the prevalent isolates from septic wounds accounting for over 50% of the isolates, followed by methicillin sensitive Staphylococcus aureus. Pseudomonas aeruginosa and Klebsiella species were isolated from a case each of PJI and hip joint infections. 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 showed that only *B. fragilis* and *P.* intermedia (one patient each) had septicemia due to anaerobic bacteremia. Diabetes and chronic infection predisposed to the anaerobic septicemia. This study therefore indicates the need for routine anaerobic blood cultures in high risk patients.

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF ANAEROBIC BACTERIA IN INFECTED BREASTS OF TURKISH WOMEN WITH LACTATIONAL MASTITIS, PERIDUCTAL AND GRANULOMATOUS MASTITIS

Bahar-Tokman, H.;*1 Taner, Z.;1 Velidedeoglu, M.;2 Goksoy, E.;2 Celik, V.;2 Demirci, M.;1 Dal, F.;2 Kucuk, Y.;2 Mete, B.;3 Yemisen, M.;3 Kocazeybek, B.;1 Gunaydın, M.;1 Kiraz, N.1

¹Istanbul University, Cerrahpasa Medical Faculty, Department of Medical Microbiology, Istanbul, Turkey

²Istanbul University, Cerrahpasa Medical Faculty, Department of *General* Surgery, Istanbul, Turkey

³Istanbul University, Cerrahpasa Medical Faculty, Department of Infectious Diseases, Istanbul, Turkey

Purpose: Anaerobic bacteria were rarely reported as infectious agents in lactational mastitis (LM), periductal mastitis (PM) and granulomateous mastitis (GM). We report the anaerobic bacteria isolated from pus samples of women diagnosed with LM, PM and GM.

Methods: Between June 2015-November 2015, anaerobic cultures were performed from pus samples collected from the infected breasts of 44 women with LM, 10 with PM and 46 with GM. Anaerobic bacteria were isolated and identified by API 20A (BioMerieux). Bacterial DNA were isolated from culture negative samples containing bacteria in Gram stained smears. PCR amplification was carried out and 1446–1515nt amplicons were sequenced by BigDye Terminator v3.1kit (Applied Biosystems, USA). DNA sequences were examined and edited using MEGA software. Susceptibility of bacteria to penicillin, amoxicillin/clavulanic acid, imipenem, clindamycin, metronidazole, and cefoxitin were investigated, with E-test (BioMerieux). The results were evaluated according to EUCAST 2015 and CLSI 2015 documents.

Results: Anaerobic bacteria were isolated from 5 LM, 1 PM and 3 GM patient's samples. *Peptostreptococcus spp.* was the most frequent isolated bacteria. Additionnaly, *Propionibacterium acnes* was isolated from 3 LM (3/5) patients. Two isolates of *Bacteroides fragilis* (2/3) and 1 isolate of *Clostridium spp* (1/3) were also isolated from patients with GM.

Anaerobic bacteria DNA was determined from 4 (8,6%) GM (3 *Propionibacterium* spp,1 *Peptostreptococcaceae bacterium*,) and from 1 LM (*Bifidobacterium breve*) patient's culture negative samples containing bacteria in Gram stained smears. Penicillin resistance was found in 3, cefoxitin resistance in 2, and clindamycine resistance was found in 1 *Peptostreptococcus spp*. Additionnaly penicillin and cefoxitine resistance was found in 2 *B.fragilis* strains.

Conclusion: The presence of anaerobic bacteria playing a primary or secondary role in mastitis should not be overlooked. The treatment protocols applied to cure these patients must also contain antibiotics effective to anaerobic bacteria.

GENOMIC COMPARISON OF MULTIPLE CAMPYLOBACTER RECTUS STRAINS

Blackburn, D.;*1 Peeler-Fletcher, S.A.;¹ Kinder, M.N.;¹ Konganti, K.;¹ Harrell, E.A.;² Threadgill, D.S.¹²

¹Texas A&M University, College Station, TX USA ²North Carolina State University, Raleigh, NC USA

Campylobacter rectus is a Gram-negative bacterium that has been linked to periodontitis. Additionally, C. rectus has been isolated from Barrett's esophagus, appendicitis, as well as oral and extraoral abscesses. Though not considered a highly pathogenic periodontal microbe, C. rectus has been implicated in bacteremia and has a potential association with pre-term births and low birth weight, indicating its importance as an emerging pathogen. We have used a comparative genomics approach using five strains of C. rectus to identify potential virulence factors. There are three major objectives for this research endeavor: (1) to provide genome sequences that will be useful for current and future C. rectus research; (2) to identify regions of variation; and (3) to discover and compare potential pathogenesis-associated genomic regions. Using Illumina, we sequenced four genomes followed by assembly into scaffolds and contigs. We compared these genomes with the C. rectus 33238 strain obtained from NCBI. Genomic comparisons have demonstrated conserved regions and regions displaying missing or additional genes in several strains. We have noted several conserved secretion systems (including flagellar/type III) and these are being further examined. Specifically, we have identified complete sets of type IV secretion system (T4SS) genes in half of our strains. T4SS are typically used for DNA uptake or transfer, but can also be used for delivering proteins. Furthermore, we have identified type VI secretion systems in all of our sequenced strains. T6SS are more diverse in their function than T4SS; though, their importance in bacterial virulence has been repeatedly documented in enteric organisms. However, there is no known role of T6SS in oral pathogens. This research endeavor has the potential to shine new light on the C. rectus species at a genetic level using whole genome analysis. Additionally, current and future work will determine functionality of T4SS and T6SS in C. rectus leading to a more comprehensive understanding of its pathogenesis.

EFFECT OF PREVIOUS BETA-LACTAM THERAPY ON RESISTANCE TO BETA-LACTAM ANTIBIOTICS IN GRAM NEGATIVE BACTERIA ISOLATED FROM CASES OF CHRONIC SUPPURATIVE OTITIS MEDIA

Egwari, L.O.;*1 Nwokoye, N.N.;2 Olubi, O.O.3

*¹Department of Biological Sciences, College of Science and Technology, Covenant University, Canaanland, Ota, Ogun State, Nigeria ²National Tuberculosis Reference Laboratory, Microbiology Division, Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria ³Department of Ear, Nose and Throat, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

Resistance to β-lactam antibiotics is on the increase and even extending to the β -lactam- β -lactamase combination. In this study, we report the effect prior β -lactam therapy will have on the resistant pattern of anaerobic Gram negative bacilli isolated from chronic suppurative otitis media. In a randomized clinical trial and laboratory investigations, 135 patients were monitored over a period of six months that met the criteria of the study, both for clinical response to treatment and bacteriological cure. The patients were in two categories; those that were on β -lactam therapy (63) and those on other antibiotic therapy (28) pre- and post-enrollment into the study. The control group consisted of 21 patients with no antibiotic history 6 months prior to the study and were treated only following culture and antibiotics sensitive test results. The 55 β -lactamase producing strains comprising Prevotella (28), Bacteroides (24), and Fusobacterium (3) were distributed as follows; group on β-lactam antibiotics (44, 80%), other antibiotics group (9, 16.4%), and control (2, 3.6%). Prevotella melaninogenica (18, 32.7), Bacteroides thetaiotaomicron (15, 27.3%), P. intermedia, and B. ureolytica (7, 12.7% each) accounted for a greater proportion of the β -lactamase producing strains, in addition to being the more frequently isolated bacteria. Only 9 of the β-lactamase producing isolates had plasmid and showed resistance to amoxicillin and cefotaxime, and curing of the bacteria of the plasmid did not alter the resistant pattern. The study showed that while β -lactam antibiotic therapy may induce resistance to this class of antibiotics, β-lactamase production does not solely account for the resistance.

COLORECTAL CANCER AND FUSOBACTERIUM NUCLEATUM INFECTION

Flynn, K.J.;* Schloss, P.D. Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI USA

Fusobacterium nucleatum is an oral microbe and periodontal pathogen in humans. In some cases, F. nucleatum migrates to the gastrointestinal tract and contributes to the progression and onset of colorectal cancer (CRC). CRC is the second deadliest cancer in the United States, and the lifetime risk of developing CRC is about 5%. Recent findings have shown that development and progression of CRC is correlated with changes in the structure of the gut microbiome. Although *F. nucleatum* is commonly isolated from carcinoma tissue samples and not healthy individuals, it is unknown how the presence of *F. nucleatum* in the gut microbial community modulates the colonic environment to contribute to carcinogenesis. We hypothesize that the composition of the gut microbial community directly affects the ability of F. nucleatum to stably colonize and contribute to colorectal carcinogenesis. To identify colonization factors that may promote F. nucleatum colonization, we treated C57/BL6 conventional mice with either cefeparazone or the inflammatory agent Dextran Sodium Sulfate (DSS) prior to infection. Mice were challenged with one 108 CFU dose of F. nucleatum. Stool samples were obtained daily for four days. Mice were sacrificed at 48h and 96h and necropsy done to obtain cecum, cecal contents, and colon tissue samples. DNA was extracted from each sample and 16SrRNA sequencing was performed to determine the structure of the bacterial community and F. nucleatum abundance. Preliminary data suggests F. nucleatum is primarily found in tissue mucosa samples at 48 and 96 hours and less abundant in stool communities. Additionally, both DSS-induced inflammation and cefeperezone treatment promote *F. nucleatum* colonization. Ongoing experiments will assess the long-term stability of *F. nucleatum* in this model and the potential to affect tumorigenesis. Further, we will be able to ascertain if loss of specific community members promotes colonization through network analysis. Delineating the contribution of the commensalturned-pathogen (F. nucleatum) and the host microbiome will hopefully result in targeted therapies for the treatment of bacterial-induced colorectal cancer.

ANTIBIOTIC SUSCEPTIBILITY PROFILES OF RECENT EUROPEAN ANAEROBES: TEST 2014-2015

Hackel, M.;*¹ Bailey-Person, M.;¹ Sahm, D.;¹ Leister-Tebbe, H.²
¹International Health Management Associates, Inc., Schaumburg, IL USA
²Pfizer Inc., Collegeville, PA USA

Objective: The Tigecycline European Surveillance Trial (TEST) has monitored susceptibility patterns of select anaerobic bacteria since 2004. In this study we evaluated tigecycline and six comparator compounds against recent European anaerobic isolates.

Methods: 2,474 anaerobic pathogens (1,443 gram negative isolates, 1,031 gram positive isolates) were collected from 2014-2015 from 19 sites in 8 countries in Europe (Belgium, Czech Republic, France, Germany, Hungary, Italy, 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 EUCAST (clindamycin [CLI], meropenem [MEM], metronidazole [MTZ], penicillin [PEN], and piperacillin-tazobactam [TZP}), CLSI (cefoxitin [FOX]), and FDA (tigecycline [TGC]) breakpoints.

Results: %S for *B. fragilis* group was: MTZ, 99.9, MEM, 94.4; TGC, 90.8; TZP, 85.9; FOX, 77.2; CLI, 62.7. %S for *Prevotella* spp. was: MTZ, 100, MEM, 100; TZP, 99.8; TGC, 99.2; FOX, 99.0; CLI, 70.0. %S for anaerobic cocci was: TGC, 100; MTZ, 99.7, MEM, 99.5; TZP, 97.4; CLI, 83.2; PEN, 88.3. %S for *Clostridium* spp. was: MTZ, 100; TGC, 99.8; MEM, 97.4; TZP, 93.8; CLI, 82.5; PEN, 60.9.

Conclusions: Tigecycline, metronidazole, and meropenem showed excellent *in vitro* activity against all anaerobic organisms isolated from European hospitals, with >90% susceptible. Clindamycin, penicillin, and cefoxitin exhibited lower activity overall, with only 65.1% of gram negative isolates susceptible to clindamycin. Piperacillin-tazobactam was less active against the *B. fragilis* group organisms. 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.

EVALUATION OF TWO DIFFERENT SYSTEMS INSTALLED ON VITEK MS SYSTEM FOR IDENTIFICATION OF CLINICALLY ISOLATED ANAEROBIC BACTERIA

Hayashi, M.;* Tanaka, K. Division of Anaerobe Research, Life Science Research Center, Gifu University, Gifu, Japan

Background: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique is expected to be a powerful tool for identification of many microorganisms isolated from clinical specimens. VITEK MS (bioMerieux, France) is one of the major MALDI-TOF MS systems specialized for this purpose. "VITEK MS plus" is equipped with two different data analytical systems: VITEK MS research-use-only (RUO) system named SARAMISTM and the *in vitro* diagnostic (IVD) system named MylaTM.

In this study, we carried out a comparative study of SARAMISTM versus $Myla^{TM}$ in their performances for identification of anaerobic bacteria.

Study: In present study, 295 clinical isolates belong to 13 genera and 29 species of anaerobic bacteria were assessed by VITEK MS plus with SARAMISTM and MylaTM. All measurements were performed on a VITEK MS plus according to the manufacture's instructions. When discrepancies in identification were observed between the SARAMISTM and MylaTM, or no identification achieved in both, identification of the strains were reconfirmed by 16S rRNA gene sequence analysis.

Result: SARAMIS[™] correctly identified 98.9% of strains (292/295), whereas Myla[™] correctly identified 78.3% of the strains (231/295). However, Myla[™] showed better performance than SARAMIS[™] for differentiation of closely related species in *Bacteroides* and identification of *Peptoniphilus asaccharolyticus*. Both SARAMIS[™] and Myla[™] could not identify 3 *Bacteroides* strains.

Conclusion: Combined use of SARAMISTM and $Myla^{TM}$ has the potential to improve performance of VITEK MS for identification of anaerobic bacteria.

INVESTIGATION OF THE ANTIBIOTIC RESISTANCE GENE CONTENT OF INTESTINAL NORMAL FLORA BACTEROIDES STRAINS USING A NOVEL CHROMOGENIC AGAR FOR THE ISOLATION

Sóki, J.;¹ Jeverica, S.;² Tierney, D.;³ Perry, J.D.;³ Nagy, E.;*¹ Urbán, E.¹ Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

²Institute for Microbiology and Immunology, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

³Microbiology Department, Freeman Hospital, Newcastle upon Tyne, UK Participating members from ESGAI: Wybo I., Belgium, Ulger N., Turkey, Stingu C-S., Germany

Objective: In a previous ESGAI study the antibiotic susceptibilities and resistance gene contents of European clinical *Bacteroides fragilis* group strains were screened. (1, 2) This time our aim was to evaluate the level of antibiotic resistance and some of the resistance genes among normal faecal flora isolates obtained from different European countries.

Methods: A newly developed selective agar (Chromogenic *Bacteroides* agar, CBA) has been introduced to isolate *B. fragilis* group strains in five European countries (Belgium, Germany, Hungary, Slovenia, and Turkey). Identification of the 243 isolates was carried out by MALDI-TOF MS (Bruker, Daltonic, Germany). Antibiotic (ampicillin, cefoxitin, meropenem, clindamycin, metronidazole, moxifloxacin, tetracycline, tigecycline, and chloramphenicol) susceptibilities were tested by agar dilution method. The resistance genes (*cepA*, *cfxA*, *cfiA* and *ermF*) were detected by RT-PCR as described previously. (2)

Results: The isolated 243 *Bacteroides / Parabacteroides* strains belonged to 16 different species (high confidence species identification was achieved) and there were only 3 isolates, which were identified by the MS method only on genus level. MIC distributions of the antibiotics were similar as observed for clinical isolates previously, however for those antibiotics with medium prevalence of resistance (10-40%) higher resistance rates were obtained in the case of normal flora isolates (cefoxitin, clindamycin, moxifloxacin). Interestingly the presence of the *cfxA* and *ermF* genes displayed even higher prevalence compared with the MIC data of cefoxitin and clindamycin. No resistance was observed for metronidazole and meropenem, however 4 of the 25 *B. fragilis* isolates were *cfiA* positive. Differences among the prevalence of the tested resistance genes were observed between the *B. fragilis* isolates and the non-fragilis *Bacteroides* strains.

Conclusion: Further studies will follow to see the prevalence of the other known resistance genes among the normal flora *Bacteroides* isolates.

^{1.} Nagy, E., Urbán, E., Nord, C.E. on behalf of the ESGRAB. Antimicrobial susceptibility of *Bacteroides* fragilis group isolates in Europe 20 years of experience. *Clinical Microbiology and Infection* 2011; 17(3): 371-379.

2. Eitel, Z., Sóki, J., Urbán, E., Nagy, E. The prevalence of antibiotic resistance genes in *Bacteroides fragilis* group strains isolated in different European countries. *Anaerobe* 2013; 21: 43-49

DETECTION AND PRELIMINARY CHARACTERIZATION OF A NOVEL MOBILIZABLE TRANSPOSON, MTnBf8, HARBORING A CHROMOSOMAL nimB GENE

Sóki, J.; Nagy, E.;* Urbán, E. Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

Objective: We aimed to determine the genetic context of the chromosomal *nimB* gene carried by several international *Bacteroides* strains, and with that, to find some experimental clue for the scare spread of metronidazole resistance.

Methods: Next-generation sequencing was applied for genome sequence determination of *nimB*-positive *Bacteroides* strains, and we used genome handling and analysis programs. For exact localization of the ends and transferability of the MTn*Bf8* transposon we applied outward-oriented PCR and conjugation experiments.

Results: The genomic sequence of B. fragilis BF8 has been determined which consisted of one scaffold with 5 contigs and 5238821 nt in length. It contained 4373 predicted ORFs and 96 RNA genes. The nimB gene was in one copy and two NBU-type integrase genes could be localized downstream (13.6 and 2.4 kb) while a Ser-tRNA sequence could be found upstream (2.4 kb). This implicated that the *nimB* gene might be on a mobilizable transposon. By means of PCR detection of the covalently-closed circular intermediate, the final sequence spam of the transposon was 16626 nt. The ends were downstream the second NBU-type integrase (attL) and in the SertRNA (attR) which harbored 22 nt complete homologies (ttgcggagagacagg... tcgaacc). Besides the NBU-type integrases, the nimB and IS1186 sequences the transposon harbored 12 other ORFs which could code virulence factors (RhaM and a fusobacterial DKNYY motif protein), an efflux pump (EmrE), possible mobilization proteins (DUF3084 and P-loop NTPase), a topoisomerase primase, homologs of other proteins with domains of unknown function (DUF1016, DUF3853, and DUF4386), or hypothetical proteins (3). The conjugal transfer of the *nimB* gene and the metronidazoleresistant phenotype from two strains (B. fragilis BF8 and KSB-R) was unsuccessful to 3 different *B. fragilis* hosts.

Conclusion: We were able to determine the carrying genetic element of the chromosomal *nimB* element (MTn*Bf8*), but could not detect its conjugal transfer. This latter finding can explain the low prevalence or the disappearance of the metronidazole resistance experienced nowadays.

EPIDEMIOLOGY OF ANTIBIOTC RESISTANCE OF CLINICALLY RELEVANT *BACTEROIDES FRAGILIS* GROUP ISOLATES IN HUNGARY

Sarvari, P.K.;¹ Nagy, E.;*¹ Miszti, C.;² Sóki, J.;¹ Urbán, E.¹

¹Department of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

²Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Objective: We set out to determine the antibiotic susceptibility of clinically relevant *Bacteroides* isolates in 2 large university clinical microbiology laboratory of Hungary.

Methods: 200 *Bacteroides fragilis* group isolates have been included so far which were isolated in routine diagnostics in university medical centres of Debrecen and Szeged, Hungary. The isolates were identified by MALDI-TOF MS and the antibiotic susceptibilities (ampicillin, amoxicillin/clavulanic acid, cefoxitin, meropenem, clindamycin, moxifloxacin, metronidazole, tetracycline, tigecycline, chloramphenicol) were recorded by agar dilution. The *B. fragilis* strains were classified by their MALDI-TOF spectra to belong the genetic divisions I (cfiA-negative) and II (cfiA-positive).

Results: The most common species was *B. fragilis* (61% of the isolates from Debrecen and 63% of the isolates from Szeged were *B. fragilis*). The following cumulative resistance rates were obtained in Debrecen and Szeged: 100% and 100% for ampicillin, 21% and 6% for amoxicilin/clavulanic acid, 6% and 13% for cefoxitin, 7% and 12% for meropenem, 25% and 34% for clindamycin, 16% and 15% for moxifloxacin, 1% and 0% for metronidazole, 60% and 60% for tetracycline, 0% and 0% for tigecycline, 0% and 0% for chloramphenicol. 19 meropenem resistant isolates were found of which 2 were *B. ovatus* and 1 was *B. thetaiotaomicron*, and we encountered 16 Division II *B. fragilis* strains.

Conlusion: As compared with previous European data, slight increases in resitance rates were found for amoxicillin/clavulanic acid and moxifloxacin, decreasing rates were found for cefoxitin and no changes in the available data for ampicillin, clindamycin, metronidazole, tigecycline. Another 200 *Bacteroides* isolates from 2 other major Hungarian university medical centres are involved in this survey, the investigations are in progress currently.

ANTIMICROBIAL SUSCEPTIBILITY OF PROPIONIBACTERIUM ACNES ISOLATED FROM ECUADORIAN PATIENTS WHO WERE UNDER ANTIMICROBIAL THERAPY

Solís, M.B.,*1 Velasco, N.;1 Gonzalez, C.,2 Dressendorfer, L.M.,1 Zurita, J.,12 Pontificia Universidad Católica del Ecuador, Medicine School, Quito, Ecuador

²Bioemedical Research Unit, Zurita&Zurita Laboratorios, Quito Ecuador

Background. Acne vulgaris is a common skin disease affecting all ages. Antibiotics remain the most common prescribed agent for the treatment of acne.

Objective. The purpose of this article is determining the incidence of resistance to *Propionibacterium acnes* in patients who had received topical and oral antibiotics used in treating acne vulgaris. **Methods.** During July to August 2015, skin swabs were collected from 129 patients with acne vulgaris then attending the dermatology department of a hospital in the city of Quito. Patients are under antimicrobial therapy less than twelve weeks. Anaerobic collection, transport, and manipulation of culture isolates were performed according to conventional methods. The strains were identified by MALDITOF. A commercially panels for anaerobic susceptibility; the Anaerobe Sensititre panel (ANO2, Thermo Fisher) were used.

Results. 129 patients were recruited. 62% was female and 38% male. The ages were between 13 and 38 years being the group of 19-24 years most prevalent. 100 patients had received twelve weeks or less tetracycline, 25 had received macrolides and 4 both. *Propionibacterium* was isolated in 71 of 129 patients; 65 were identified as *P. acnes* 3 as *P. granulosum*, and 3 as *P. acidifaciens*. No resistant strains were detected to Imipenem Meropenem and Mezlocillin. 1.4% to Amoxicillin-clavulanate, Ampicillin-sulbactam, Piperacillin, and Piperacillin-tazobactam. 4.2% to Ampicillin, Penicillin, Cefotetan. 9.8% to Tetracycline. 11.2% to Clindamycin and Cefoxitin, and 100% resistance to Metronidazole.

Conclusions. As long as antibiotics are used, drug resistance will remain a challenge. To prevent the development of antibiotic resistance, the practice of prescribing antibiotics for treatment of acne must be examined carefully. Susceptibility among anaerobes differs from species to species and also from region to region. Improper use of antibiotics in the dermatological setting needs to be evaluated to prevent the increasing prevalence of antibiotic resistance.

ANTIMICROBIAL SUSCEPTIBILITIES FOR 331 STRAINS OF PREVOTELLA SPECIES ISOLATED IN JAPAN

Yamagishi, Y.;* Suematsu, H.; Nishiyama, N.; Koizumi, Y.; Mikamo, H. Department of Clinical Infectious Diseases, Aichi Medical University Hospital, Aichi, Japan

Objectives: To determine antimicrobial susceptibilities for clinical isolates of *Prevotella* species isolated in Japan.

Methods: We investigated antimicrobial susceptibilities for 331 strains of *Prevotella* species isolated in Aichi Medical University Hospital, Japan, from January 2011 to October 2015.

Results: The dominant *Prevotella* species isolated was *Prevotella bivia* with 24%, following Prevotella melaninogenica with 15% and Prevotella intermedia with 15%. The dominant specimens isolated Prevotella species specimen was genital discharge with 26.3%, following ascites in abdominal or pelvic cavity with 19.6%, oral secretion with 11.4%, and specimens from head and neck with 11.4%. Carbapenems showed the best activity against *Prevotella* species, following piperacillin/tazobactam and sulbactam/cefoperazone. Susceptible rates of moxifloxacin against P. intermedia, P. melaninogenica and P. bivia remained 67.3%, 54.9% and 43.0%, respectively. Susceptibilities of moxifloxacin against *P. bivia* were significantly lower, compared susceptibilities of moxifloxacin against P. intermedia (p<0.05). Susceptibilities of moxifloxacin against P. melaninogenica were significantly reduced, compared with 2013 or later (p<0.05). Susceptibilities of clindamycin against *P. intermedia*, *P.* melaninogenica and P. bivia were 72.5%, 62.7% and 65.8%, respectively. No significant changes were observed during investigation period.

Conclusion: Since antimicrobial resistances of *Prevotella* species have been going up, continuous surveillance would be needed in Japan.

ANTIMICROBIAL SUSCEPTIBILITIES FOR 67 STRAINS OF PEPTOSTREPTOCCUS ANAEROBIUS ISOLATED IN JAPAN

Mikamo, H.; Yamagishi, Y.;* Suematsu, H.; Nishiyama, N.; Koizumi, Y. Department of Clinical Infectious Diseases, Aichi Medical University Hospital, Aichi, Japan

Objectives: To investigate the antimicrobial susceptibilities for clinical isolates of *Peptostreptococcus anaerobius* in Japan.

Methods: We investigated antimicrobial susceptibilities for 67 strains of *P. anaerobius* isolated in Aichi Medical University Hospital, Japan, from January 2012 to October 2015.

Results: Clinical isolates of *P. anaerobius* were isolated from department of gynecology with 50.7%, following dermatology with 14.9%. The dominant specimens isolated *P. anaerobius* were genital discharge with 43.9% Piperacillin/tazobactam, cefmetazole, imipenem, and meropenem showed good antimicrobial activities against *P. anaerobius*. Susceptible rates of ampicillin, amoxicillin/clavulate acid, clindamycin, and moxifloxacin were 76.1%, 77.6%, 74.6%, and 38.8%, respectively. The MIC₉₀s for anti-anaerobic quinolones, moxifloxacin, garenoxacin and sitafloxacin, were 8, over $16\,\mu$ g/mLand $0.5\,\mu$ g/mL, respectively.

Conclusion: Since resistant rates of anti-anaerobic quinolones for *P. anaerobius* have been going up, continuous surveillance on antimicrobial resistance would be needed in future.

THE ROLE OF ANAEROBIC BACTERIA IN POLYMICROBIAL NECROTIZING WOUND INFECTIONS

Zhao-Fleming, H.H.;* Rumbaugh, K.P. Texas Tech University Health Sciences Center, Department of Surgery Lubbock, TX USA

Skin and soft tissue infections can manifest in a variety of ways, ranging from a self-resolving abscess to a rapidly spreading necrotizing soft tissue infection (NSTI). While an abscess may require only minor medical intervention, if treatment is needed at all, an NSTI is a medical emergency, requiring immediate surgical intervention. NSTIs can either be monomicrobial or, more commonly, polymicrobial. Monomicrobial NSTIs generally involve well-studied pathogen such as Streptococcus pyogenes or Clostridium perfringens. Polymicrobial NSTIs, however, are not as well understood. *Staphylococcus aureus* (*Sa*), a facultative anaerobe, is the most commonly isolated pathogen in polymicrobial NSTIs, but it is also the most commonly isolated pathogen in abscess infections. The other microbial and host contributions that contribute to the development of NSTIs are not well studied. We hypothesize several different causes, one of which is the involvement of anaerobic bacteria, which are difficult to culture and thus often missed in pathogen isolation studies. <u>In this study, we propose</u> to better understand the role of anaerobic bacteria in the pathology of necrotizing infections. In order to further elucidate the contribution of anaerobic bacteria in the development of NSTIs, we introduced a commonly isolated wound anaerobe, Bacteroides fragilis (Bf), with Sa into in vitro and *in vivo* wound models. We asked how does *Bf* affect the growth and virulence of Sa. Using an in vitro wound-like model, our preliminary data showed that in aerobic conditions, Bf required Sa to survive. Using our in vivo mouse abscess model, we show that the presence of Sa and Bf resulted in a worsened disease pathology than either bacteria alone. Based on our data, we conclude that Bf is a significant contributing factor in NSTI disease progression. Future research efforts will be directed towards solidifying current data, elucidating the underlying mechanisms of this potential synergy in virulence, and understanding if a similar phenomenon occurs with other common wound co-infections such as those involving *Finegoldia* magna.

103

THE ROLE OF ANAEROBIC BACTERIA IN POLYMICROBIAL INFECTIONS

Zhao-Fleming, H.H.;* Rumbaugh, K.P. Texas Tech University Health Sciences Center, Department of Surgery Lubbock, TX USA

Skin and soft tissue infections can manifest in a variety of ways, ranging from a self-resolving abscess to a rapidly spreading necrotizing soft tissue infection (NSTI). While an abscess may require only minor medical intervention, if treatment is needed at all, an NSTI is a medical emergency, requiring immediate surgical intervention. NSTIs can either be monomicrobial or, more commonly, polymicrobial. Monomicrobial NSTIs generally involve well-studied pathogen such as Streptococcus pyogenes or Clostridium perfringens. Polymicrobial NSTIs, however, are not as well understood. Staphylococcus aureus (Sa), a facultative anaerobe, is the most commonly isolated pathogen in polymicrobial NSTIs, but it is also the most commonly isolated pathogen in abscess infections. The other microbial and host contributions that contribute to the development of NSTIs are not well studied. We hypothesize several different causes, one of which is the involvement of anaerobic bacteria, which are difficult to culture and thus often missed in pathogen isolation studies. In order to further elucidate the contribution of anaerobic bacteria in the development of NSTIs, we introduced a commonly isolated wound anaerobe, *Bacteroides fragilis* (Bf), with Sa into in vitro and in vivo wound models. We asked how does Bf affect the growth and virulence of Sa. Using an in vitro wound-like model, our preliminary data showed that in aerobic conditions, Bf required Sa to survive. Using our *in vivo* mouse abscess model, we show that the presence of Sa and Bf caused dermonecrosis 100% of the time while Sa alone only caused dermonecrosis about 50% of the time. Future research efforts will be directed towards solidifying current data, elucidating the underlying mechanisms of this potential synergy in virulence, and understanding if a similar phenomenon occurs with other common wound co-infections such as those involving Finegoldia magna.

1315	POSTER SESSION I: NON-DIFFICILE CLOSTRIDIA	
PI-17	Comparison of <i>Clostridium perfringens</i> Toxin Genes in Isolates from Broiler Chickens Deemed Healthy, and Those with Clinical and Subclinical Enteritis	106
	Brown, J.C.S.;* Dixon, R.A.	
PI-18	Mepacrine Reduces Caco-2 Cell Death Caused by <i>Clostridium</i> perfringens Enterotoxin (CPE)	107
	Freedman, J.C.;* McClane, B.A.	
PI-19	Chronic Osteomyelitis Caused by Clostridium hydrogeniformans Hirai, J.;* Yamagishi, Y.; Sakanashi, D.; Suematsu, H.; Kinjo, T.; Fujita, J.; Mikamo, H.	108
PI-20	Intestinal Pathology in Goats Challenged with <i>Clostridium</i> perfringens Type D Wild-Type Strain CN1020 and its Genetically Modified Derivatives	109
	Morrell, E.L.;* Garcia, J.P.; Beingesser, J.; Adams, V.; Rood, J.I.; Uzal, F.A.	
PI-21	Comparison of Penicillin-Binding Proteins in Beta-Lactam Resistant Strains of Clostridium perfringens	110
DI 22	Park, M.;* Rafii, F.	
PI-22	Fluoroquinolone Resistance Selection Affects Sugar Transport Osmotolerance of <i>Clostridium perfringens</i> Park, M.; Rafii, F.*	111
PI-23	Reclassification of Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme, and Clostridium saccharogumia to a Novel Genus, Flintia gen. nov. Saavedra, L.;* Finegold, S.M.; Lawson, P.A.	112
PI-24	Comparison of Growth of <i>Clostridium perfringens</i> on Different <i>in vitro</i> Digested Broiler Diets	113
	Saleem, G.;* Sparks, N.; Houdijk, J.	
PI-25	Characterization of Clostridium Isolates that Cause Flooding-Associated Soft Rot of Sweet Potato DaSilva, W.L.: Yang, K.T.:* Pettis, G.S.: Clark, C.A.	114

COMPARISON OF *CLOSTRIDIUM PERFRINGENS* TOXIN GENES IN ISOLATES FROM BROILER CHICKENS DEEMED HEALTHY, AND THOSE WITH CLINICAL AND SUBCLINICAL ENTERITIS

Brown, J.C.S.;* Dixon, R.A. University of Lincoln, School of Life Sciences, Lincoln, United Kingdom

Introduction: Clostridium perfringens is involved in the pathogenesis of the avian disease necrotic enteritis (NE). It occurs both pathogenically, and as part of the commensal bacteria of the avian environment, and the gut microbiota of chickens. *C. perfringens* has a complex arsenal of toxins, though a specific toxin that is directly causative of the disease has yet to be elucidated.

Methods: 60 chickens were culled from varied UK commercial sites with identical biocontrol measures, but with different health status. Birds were necropsied, with the gastrointestinal tract (GIT) removed immediately for sampling along with the gizzard and crop content. Samples were streaked onto tryptose sulphite cycloserine agar, and confirmed by biochemical testing. Isolates were subject to toxin typing by polymerase chain reaction (PCR) for the presence of *cpa*, *cpb*, *iA*, *etx*, *cpb2*, *cpe*, *pfoA*, *colA*, *netB*, *tpeL*, *becA*, and *becB* toxin genes. Isolates were also subjected to PFGE analysis.

Results: 31.25% (15/48) of the birds were diagnosed with subclinical NE and 10.42% (5/48) birds were found to have clinical NE when excluding the control group which were confirmed as healthy. *C. perfringens* was recovered from 41.7% of crop, 50% of gizzard, 16.7% of duodenum, 45% of jejunum, 40% of ileum and 75% of ceca samples. All birds with NE were found to have *C. perfringens* in their GIT. All samples were found to be type AAll birds with either clinical or subclinical NE were found to have the *cpb2* gene. PFGE profiles showed a high genetic diversity among the isolates.

Conclusion: *C. perfringens* was demonstrated to colonise UK broiler chickens both in NE and as part of the commensal bacteria within poultry environments. The bacterium was recovered extensively from the crop, gizzard, duodenum, jejunum, ileum and caeca with different toxin profiles identified at each site. It was found *cpb*2 was frequently recovered from birds with NE, but the *netB* was not detected.

MEPACRINE REDUCES CACO-2 CELL DEATH CAUSED BY CLOSTRIDIUM PERFRINGENS ENTEROTOXIN (CPE)

Freedman, J.C.;* McClane, B.A. Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA USA

Clostridium perfringens is a Gram-positive, spore-forming, anaerobe that causes a number of medically important diseases in humans and animals. C. perfringens strains producing the C. perfringens enterotoxin (CPE) cause over 1 million cases of diarrheal food poisoning in the United States, at a cost of 400 million USD per year. Additionally, CPE-positive strains of *C. perfringens* cause 5-20% of all cases of antibiotic-associated diarrhea (AAD), a disease that presents with recurring rounds of often severe diarrhea. As such, the investigation of potential therapies for CPE-mediated disease is warranted. CPE is a pore-forming toxin, which binds to claudin receptor proteins on the tight junctions of host cells and oligomerizes to form a hexameric pore that inserts into the membrane and causes an influx of Ca²⁺ into the cell, leading to the activation of cell death pathways. A previous study demonstrated that mepacrine is able to block the activity of the CPE pore in lipid bilayers in the absence of claudin receptor proteins. In this study, we sought to determine if mepacrine can prevent CPE-induced cellular cytotoxicity. For this work, we used Caco-2 cells, a claudin-producing, naturally CPE-sensitive cell line. Interestingly, we found that mepacrine reduces CPE-induced cytotoxicity of Caco-2 cells in a dose-dependent manner, with higher mepacrine doses nearly eliminating Caco-2 cytotoxicity, indicating the potential utility of mepacrine as a therapeutic for CPE-mediated disease. On-going studies are determining whether mepacrine can prevent CPE-induced cytotoxicity without pretreatment, if mepacrine can prevent cytotoxicity at higher CPE doses, and whether mepacrine prevents CPE-binding and/or CPE pore activity on Caco-2 cells. Future studies will investigate the utility of mepacrine use in vivo to protect against CPE-induced intestinal damage.

CHRONIC OSTEOMYELITIS CAUSED BY CLOSTRIDIUM HYDROGENIFORMANS

Hirai, J.;*1,2,3 Yamagishi, Y.;1,2 Sakanashi, D.;2 Suematsu, H.;2 Kinjo, T.;3 Fujita, J.;3 Mikamo, H.1,2

¹Department of Clinical Infectious Diseases, Aichi Medical University, Nagakute, Japan

²Department of Infection Control and Prevention, Aichi Medical University Hospital, Nagakute, Japan

³Department of Infectious, Respiratory, and Digestive Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan

Introduction: *Clostridium* species is an important pathogen, causing many infections, however, osteomyelitis, due to this species, is quite rare. Herein, we report a case of chronic osteomyelitis caused by *C. hydrogeniformans*.

Case: A previously healthy 18-year-old male was admitted to the emergency room complaining of right arm injury due to traffic accident. He had fracture of his right ulna, and surgical debridement and systemic administration of antibiotics were immediately performed. Although he was discharged on 7th hospital day, he had a fever and pus discharge was observed at surgical site on 30th day after discharge. Because high-resolution computed tomography showed subcutaneous abscess and osteomyelitis was suspected, emergency operation was performed. Although aerobic culture was negative, anaerobic incubation of the bone tissue and pus cultured gas producing Gram-positive rod three days after surgery. The organism was identified as *C. hydrogeniformans* with 99% homology by 16S rRNA gene sequence analysis. Antibiotics against *C. hydrogeniformans* were continued for 90 days until bone adhesion was observed. No recurrence has occurred.

Discussion: *C. hydrogeniformans* was identified from chlorinated solvent-contaminated groundwater by 16S rRNA gene sequence analysis as novel species of *Clostridium* genus by Bowman KS, *et al.* in 2010. Infectious disease due to this species has not been reported so far. We suspected the infection source of this case was the drain of the road where he fell when he got injured in a traffic accident.

Conclusion: To the best of our knowledge, this is the first reported case of infectious disease caused by *C. hydrogeniformans*. If anaerobic culture method becomes more common in clinical setting, this pathogen might be detected more often in traumatic cases.

INTESTINAL PATHOLOGY IN GOATS CHALLENGED WITH CLOSTRIDIUM PERFRINGENS TYPE D WILD-TYPE STRAIN CN1020 AND ITS GENETICALLY MODIFIED DERIVATIVES

Morrell, E.L.;*¹ Garcia, J.P.;¹ Beingesser, J.;¹ Adams, V.;² Rood, J.I.;² Uzal, F.A.¹ California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, Davis, CA USA

²Australian Research Council Centre of Excellence in Structural and Functional Microbial Genomics, Department of Microbiology, Monash University, Victoria, Australia

Clostridium perfringens type D is the causative agent of enterotoxaemia in sheep, goats and cattle. While in sheep and cattle, the disease is mainly characterized by neurological clinical signs and lesions, goats with type D enterotoxemia frequently present alterations of the alimentary system. Epsilon toxin (ETX) is the main virulence factor of *C. perfringens* type D, although the role of ETX in intestinal lesions in goats with type D enterotoxemia has not been fully characterized. We evaluated the contribution of ETX to *C. perfringens* type D enteric pathogenicity in an intraduodenal challenge model in young goats, using the virulent C. perfringens type D wild type strain CN1020 (WT; n=3), an isogenic ETX null mutant (etx mutant; n=3), a strain where the etx mutation had been complemented (etx complemented; n=6) and sterile, non-toxic culture medium (control; n=2). A score system of 0 (no lesions) to 4 (severe lesions) was used to evaluate microscopic lesions in the small and large intestine of each animal and an average score was calculated for each group. Both ETX-producing strains induced extensive enteritis and/or colitis. The average of the overall severity score was 3.91±0.7 and 3.09±1.2 in the WT and etx complemented groups, respectively. By contrast, goats inoculated with the *etx* mutant or sterile, non-toxic culture medium, develop minimal or no detectable intestinal lesions, with average severity scores for intestinal lesions of 0.5 ± 0.7 and 0.4 ± 0.5 , respectively. These results confirm that the enteric effects observed in goats with type D enterotoxemia are dependent on ETX production.

108

COMPARISON OF PENICILLIN-BINDING PROTEINS IN BETA-LACTAM RESISTANT STRAINS OF CLOSTRIDIUM PERFRINGENS

Park, M.;* Rafii, F. Division of Microbiology, National Center for Toxicological Research, FDA, Jefferson, AR USA

Decrease in the affinity of various β-lactams for penicillin-binding proteins (PBP) is considered one of the reasons for resistance to β -lactams in *Clostridium perfringens.* Variations in the sequences of PBP that may affect the affinity of different β-lactams to PBP in *C. perfringens* were investigated. One chicken isolate (B45) and two bovine isolates (U30 and S36) were examined for resistance to β-lactams. Sensitivity to cefoxitin, ceftriaxone, and amoxicillin was measured by Etest. PCR was used to amplify different PBP genes, using the sequences of CPF_2218, CPF_2395, CPF_2376 and CPF_0340 from C. perfringens ATCC 13124 for designing the primers, and the amplicons were sequenced. The chicken isolate B45 was resistant to all three β-lactams. One bovine isolate (S36) was resistant to cefoxitin and ceftriaxone but sensitive to amoxicillin. The other bovine isolate (U30) was sensitive to cefoxitin but resistant to ceftriaxone and amoxicillin. The bovine and chicken isolates resistant to amoxicillin had mutations in CPF_2218, resulting in the substitution of Asn245Asp and Asn441Asp, respectively, in comparison with this gene in ATCC 13124. The PBP from the amoxicillinsensitive bovine isolate S36 did not have this alteration but had changes of Asp276Gly and Asp361Arg. There were no mutations in the sequenced sections of CPF_0340 and CPF_2395 for the cefoxitin-sensitive bovine isolate U30. Bovine isolate S36 and chicken isolate B45, resistant to cefoxitin, had mutations in CPF 2395 that resulted in the substitution of one and three amino acids, respectively. They also had mutations in CPF_0340, resulting in the substitution of three amino acids in PBP of chicken isolate B45 and of six amino acids in bovine isolate S36. An in vitro generated penicillin resistant mutant of ATCC 13124 was resistant to amoxicillin, cefoxitin, and ceftriaxone and had an alteration in CPF_2376 resulting in conversion of Glu98Gly and Thr267Ala. In conclusion, variations were observed in PBP proteins of *C*. *perfringens* that were resistant to different β-lactams. Two of the field isolates had amino acid substitutions in all three PBP examined when compared to ATCC 13124.

FLUOROQUINOLONE RESISTANCE SELECTION AFFECTS SUGAR TRANSPORT AND OSMOTOLERANCE OF CLOSTRIDIUM PERFRINGENS

Park, M.; Rafii, F.* Division of Microbiology, National Center for Toxicological Research, FDA, Jefferson, AR USA

Clostridium perfringens, an anaerobic, spore-forming bacterium, produces several toxins and is pathogenic for humans and other animals. The production of toxins in *C. perfringens* is decreased by sugars and the effect is attributed to the activities of regulatory proteins. A fluoroquinolone (gatifloxacin)-resistant mutant of C. perfringens ATCC 13124, designated 13124^{GR}, produces lower quantities of several toxins than the wild type. The effects of carbohydrates on the wild type and the mutant were compared by culturing the strains in a minimal medium containing various sugars and examining their growth after incubation under anaerobic conditions. The wild type and mutant were also examined for growth in different concentrations of sugars, salts and bile acids, and at different pH values by culturing in brain heart infusion under anaerobic conditions at 37°C. The role of trehalose in osmoprotection was compared by growing strains in different NaCl concentrations in a medium supplemented with trehalose. The genes for the *tre* locus were compared by PCR and sequencing. Quantitative real-time PCR (qRT-PCR) was used to measure transcription of the genes. In a chemically defined medium, trehalose and sucrose supported the growth of the wild type but not the mutant. Microarray data and qRT-PCR showed that putative genes for the transport of sucrose and trehalose (via a phosphoenolpyruvate-dependent phosphotransferase system) and some of the regulatory genes were downregulated in the mutant. The wild type had greater tolerance than the mutant to salts and low pH. Trehalose and sucrose enhanced the osmotic tolerance of the wild type to NaCl. This is the first study showing the involvement of a putative gene in the transport of trehalose in *C. perfringens*, its role in the protection of cells from environmental stress, and the effect of fluoroquinolone resistance selection on the transport of this disaccharide.

110

RECLASSIFICATION OF CLOSTRIDIUM COCLEATUM, CLOSTRIDIUM RAMOSUM, CLOSTRIDIUM SPIROFORME, AND CLOSTRIDIUM SACCHAROGUMIA TO A NOVEL GENUS, FLINTIA GEN. NOV.

Saavedra, L.;*1 Finegold, S.M.;2 Lawson, P.A.1

¹Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK USA

²Department of Medicine, and Department of Microbiology, Immunology, and Molecular Genetics, UCLA School of Medicine, Los Angeles, CA USA

It's been recently proposed to restrict the genus Clostridium to C. butyricum and relatives (rRNA cluster I). Such a restriction will ensure that many species will no longer be true members of this genus. *Clostridium cocleatum*, Clostridium ramosum, Clostridium spiroforme, and Clostridium saccharogumia are located in clostridial rRNA cluster XVIII and form a robust phylogenetic group based on 16S gene sequencing. In this study these organisms were subjected to a polyphasic investigation that included phenotypic and chemotaxonomic methods. C. cocleatum, C. ramosum, C. spiroforme, and C. saccharogumia were obtained from the CCUG. Cellular fatty acids were analyzed using the MIDI system. The profiles showed that all the strains' dominant fatty acids were $C_{14:0'}$, $C_{16:0'}$ and $C_{18:1 \text{ W7c}}$. Whole-cell sugar analysis was consistent between all organisms for the presence of galactose. Peptidoglycan analysis showed that *meso-2*,6-diaminopimelic acid was present as the diagnostic diamino acid. Biochemical profiles derived from API test systems were also concordant with being closely related species. According to these findings, it is proposed to create a novel genus, "Flintia" to accommodate Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme, and Clostridium saccharogumia as "Flintia cocleatum gen. nov. comb. nov.", "Flintia ramosum comb. nov.", "Flintia saccharogumia comb. nov.", and "Flintia spiroforme comb. nov." The novel genus is named in honor of the British microbiologist Harry Flint for his many contributions to anaerobic microbiology of the human gut.

COMPARISON OF GROWTH OF CLOSTRIDIUM PERFRINGENS ON DIFFERENT IN VITRO DIGESTED BROILER DIETS

Saleem, G.;*1 Sparks, N.;2 Houdijk, J.2

¹Pathology Department, University of Veterinary and Animal Sciences, Lahore, Pakistan

²Monogastric Science Research Centre, Animal and Veterinary Sciences, SRUC, Edinburgh, Scotland UK

Necrotic enteritis (NE) remains a major problem in broiler flocks. The primary cause of NE is the overgrowth of *Clostridium perfringens* type A, a normal inhabitant of the gastrointestinal tract. It has been suggested that feeding high levels of fish meal may increase NE incidence. The present study determines the proliferation of *C. perfringens* type A on *in* vitro digested feeds without or with 30% fishmeal. 3ml of digested diet supernatant was mixed with 6ml of thioglycollate medium and 1ml of C. perfringens type A culture (7.81 log₁₀ cfu). Colonies were enumerated after 3, 6, 12, and 24 hours of incubation by pour plating. Individual colonies were randomly selected and confirmed as C. perfringens using Gram staining. Initially all supernatants were assessed for the presence of *C. perfringens* and were found to be negative. C. perfringens counts averaged 8.63, 8.80, 7.83, and 7.13 log₁₀ cfu/ml after 3, 6, 12, and 24 h of incubations, respectively (s.e.d. 0.26 log₁₀ cfu/ml; P<0.001). The presence of 30% fish meal tended to increase *C. perfringens* proliferation (8.26 vs 7.93 log₁₀ cfu/ml; s.e.d. 0.18 log10 cfu/ml; P=0.084). Whilst the interaction between diet and time was not formally significant (P=0.13), fish meal effects were most pronounced after 24 h of incubation (7.60 vs 6.65 \log_{10} cfu/ml; s.e.d. 0.36 $\log 10$ cfu/ml; P<0.05). These findings support the view that high levels of dietary fish meal may assist survival of *C. perfringens*, suggestion a role of fishmeal as a predisposing factor for NE cannot be excluded.

CHARACTERIZATION OF *CLOSTRIDIUM* ISOLATES THAT CAUSE FLOODING-ASSOCIATED SOFT ROT OF SWEET POTATO

DaSilva, W.L.;¹ Yang, K.T.;*² Pettis, G.S.;^{1,2} Clark, C.A.¹
¹Department of Plant Physiology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA USA
²Department of Biological Sciences, Louisiana State University, Baton Rouge, LA USA

Production of sweetpotato in the southern United States has become increasingly hampered in recent years by a soft rot disease which develops on the edible storage roots following storms and hurricanes that result in flooding of fields. We wished to characterize the microbial pathogen(s) responsible for development of soft rot under these anaerobic conditions. To this end, tissue samples of storage roots with soft rot taken from intentionally flooded fields were streaked on nutrient dextrose agar plates containing 0.05% cysteine and incubated anaerobically. From the two distinct groups of bacteria consistently isolated from this analysis, single representatives, designated LSU-B1 and LSU-B7, were chosen for further study. In pathogenicity tests, sweetpotato storage roots inoculated with either LSU-B1 or LSU-B7 displayed severe soft rot following one week of incubation under hypoxic conditions. Both isolates also elicited soft rot symptoms when similarly tested on potato tuber, onion bulb, and carrot root. In every case, the inoculated isolates were re-isolated from the diseased tissue. Staining procedures revealed LSU-B1 and LSU-B7 to be Gram-positive endospore formers. However, the isolates were clearly distinguishable based on colony morphology, average size of vegetative cells and pectolytic activity on double-layer pectate medium, which was displayed only by LSU-B1. 16S rRNA gene sequencing revealed that LSU-B1 and LSU-B7 belong to the genus Clostridium. Within this genus, only one plant pathogenic species, Clostridium puniceum, has been described, and it was characterized as a soft rot pathogen of potato. We are currently performing comparative fatty acid analysis on LSU-B1, LSU-B7, and C. puniceum in order to learn more about the degree of relatedness among them. Overall, our results provide evidence that members of the genus Clostridium can naturally cause devastating soft rot disease on sweetpotato and potentially on a variety of other vegetable crops as well.

1315	POSTER SESSION I: GUT MICROBIOME	
PI-26	Determination of the Effect of Maillard Products on the Taxonomic Composition on the Gut Microbiota ALJahdali, N.;* Gadonna, P.; Anton-Gay, P.; Carbonero, F.	116
PI-27	Bacteroidetes and Firmicutes Numbers in Gut Microbiota of Adult Type 1 Diabetes Patients and Healthy Turkish People: Effects on Hosts TLR2 & TLR4 Gene Expression Levels	117
	Demirci, M.; Temeloglu-Keskin, E.; Cagatay, P.; Taner, Z.; Ozyazar, M.; Kocazeybek, B.; Kiraz, N.; Bahar-Tokman, H.*	
PI-28	The Mosaic of Cytochromes Expression from Bacteria to Man and Inflammation Processes	118
	Stavropoulou, E.;* Bezirtzoglou, E.	
PI-29	The Microbiota in a Model of Alzheimer's Disease, Aging, and Dietary Intervention	119
	Cox, L.M.;* Schafer, M.J.; Sohn, J.; Weiner, H.L.; Ginsberg, S.D.; Blaser, M.J.	
PI-30	The Fecal Microbiome of Dogs with Exocrine Pancreatic Insufficiency	120
	Isaiah, A.;* Parambeth, J.C.; Steiner, J.M.; Suchodolski, J.S.	
PI-31	Establishment and Development of Intestinal Microbiota in Preterm Infants of a Lebanese Tertiary Hospital	121
	Itani, T.;* Ayoub Moubareck, C.; Melki, I.; Delannoy, J.; Mangin, I.; Butel, M.J.; Karam Sarkis, D.	
PI-32	Characterization of Phenotypic and Genetic Diversity among Lachnospiraceae Isolates from the Human Gut Microbiota <i>Lau</i> , <i>J.T.;* Surette</i> , <i>M.G.</i>	122
PI-33	Gut Microbiota in Healthy Subjects and Inpatients with Clostridium difficile Infection	123
	Flecher, T.B.; Miranda, K.R.;* Secco, D.A.; Peixoto, R.S.; Rosado, A.S.; do Carmo, F.L.; de Jesus, H.E.; Antunes, L.C.M.; de Paula, G.R.; Domingues, R.M.C.P.	
PI-34	Effect of Topsoil Exposure During Lactation on Subsequent Performance and Gut Microbiota in Pigs	124
	Vo, N.;* Tsai, T.; Kim, H.; Sales, M.A.; Wang, X.; Erf, G.E.; Kegley, E.B.; van der Merwe, M.; Buddington, R.; Maxwell, C.V.; Carbonero, F.	

Posters will be presented in Poster Session I Tuesday, July 12 1315-1415.

DETERMINATION OF THE EFFECT OF MAILLARD PRODUCTS ON THE TAXONOMIC COMPOSITION ON THE GUT MICROBIOTA

ALJahdali, N.;*¹ Gadonna, P.;² Anton-Gay, P.;² Carbonero, F.^{1,3}
¹Cellular and Microbiology Program; University of Arkansas, Fayetteville, AR, USA

²Expression des Gènes et régulation Epigénétique par l'Aliment; Institut Polytechnique LaSalle, Beauvais, France

³Department of Food Science; University of Arkansas, Fayetteville, AR, USA

Maillard reaction products (MRPs) are the chemical reactions between amino acids and reducing sugar generated after the heat treatment of food. It has been reported that highly heated pellets, mice food, were proved to significantly protect against inflammation of experimental colitis in mice. The aim of this study is to determine the effect of Maillard products on the taxonomic composition of gut microbiota in mouse models of digestive diseases. In this study, mice were divided into 3 groups and given chow subjected to various heat treatments (no treatment (control), moderately heated 121° C for 30 min, and highly heated 150° C for 15 min. The fecal samples were collected and microbial DNA extracted. Illumina MiSeq sequencing was used to survey the gut microbiota by targeting the V4 region of the bacterial 16S rRNA gene. In general, microbial communities were dominated by phylum level of Firmicutes and Bacteroidetes. Sequences were analyzed with the Basespace software and Mothur. Highly heated maillard products consumption resulted in a very significant increase of Firmicutes, specifically Blautia and Faecalibacterium spp. This increase was mainly at the expense of Bacteroides spp. The consumption of moderately had more moderate effects, but an diversity increase and a slightly more significant increase of Faecalibacterium spp. were observed. These data indicate that Maillard products may have an unconventional prebiotic effect by the stimulation of Faecalibacterium and Blautia species.

BACTEROIDETES AND FIRMICUTES NUMBERS IN GUT MICROBIOTA OF ADULT TYPE 1 DIABETES PATIENTS AND HEALTHY TURKISH PEOPLE: EFFECTS ON HOSTS TLR2 & TLR4 GENE EXPRESSION LEVELS

Demirci, M.;¹ Temeloglu-Keskin, E.;² Cagatay, P.;³ Taner, Z.;¹ Ozyazar, M.;² Kocazeybek, B.; Kiraz, N.; Bahar-Tokman, H.*¹

¹Istanbul University, Cerrahpaşa School of Medecine, Department of Medical Microbiology, Istanbul, Turkey

²Istanbul University, Cerrahpaşa School of Medecine, Department of Endocrinology, Istanbul, Turkey

³Istanbul University, Istanbul Medical Faculty, Department of Biostatistics and Medical Informatics, Istanbul, Turkey

Purpose: Gut microbiota has been proposed to play an important role in the development of Type 1 diabetes. We investigated and compared the amount of *Bacteroidetes* and *Firmicutes* in the gut microbiota of Type 1 diabetes patients and in healthy controls. We measured and compared the TLR2 and TLR4 gene expression levels in persons of these two groups.

Methods: Between January-October 2014, from 53 type 1 diabetes patients (28 male and 25 female) and 53 healthy controls (28 male and 25 female) fecal samples, the log₁₀ Bacteroidetes and Firmicutes numbers per gram of feces were measured via qPCR. Also, TLR2 and TLR4 gene expression levels were investigated with qPCR from whole blood samples with EDTA collected from persons of these groups and the results were compared. IBM SPSS Statistics vers 20 was used for statistical analyses.

Results: The log₁₀ Bacteroidetes and Firmicutes numbers per gram of feces was found 11,45 \pm 0,25 and 9,31 \pm 0,57 in male, 11,65 \pm 0,35 and 9,22 \pm 0,51 in female patients. Comparing with those of healthy controls, the log₁₀ Bacteroidetes number was found statistically extremely high in male and female patients (p<0,001), the \log_{10} Firmicutes number was found statistically extremely high in male and statistically high in female (p<0,001,p=0.007) patients. Depending on these results, as expected, the TLR2 gene expression levels were found $1{,}15 \pm 0{,}07$ in male and $1{,}14 \pm 0{,}06$ in female patients. The TLR4 gene expression levels were found $1,14 \pm 0,04$ in male and 1,17± 0,04 in female patients. Comparing with those of healthy controls,TLR4 gene expression levels were found statistically extremely high in both male and female patients (p<0,001), TLR2 gene expression levels were found statistically high in male (p=0,001) and extremely high (p<0,001) in female patients. The *Firmicutes / Bacteroidetes* ratio was 0.8029 ± 0.047 in Tip 1 diabetes patients and 0.9134 ± 0.061 in healthy controls. Comparing with those of healthy controls, the *Firmicutes / Bacteroidetes* ratio was found statistically extremely low in both male and female patients groups(p<0,001).

Conclusion.We concluded that there is a two-way relationship between Tip 1 diabetes and the quantity of *Firmicutes* and *Bacteroidetes* in the gut microbiota . Especially the *Firmicutes / Bacteroidetes* ratio is the result of mental and physical dynamics existing in human body and influencing the gut microbiota.

THE MOSAIC OF CYTOCHROMES EXPRESSION FROM BACTERIA TO MAN AND INFLAMMATION PROCESSES

Stavropoulou, E.;*1 Bezirtzoglou, E.2

¹School of Medicine, Democritus, University of Thrace, Alexandroupolis, Greece

²Faculty of Agricultural Development, Department of Food Science and Technology, Laboratory of Microbiology, Biotechnology and Hygiene, Democritus University of Thrace, Orestiada, Greece

Cytochromes are located in many different tissues of the human body. They are found in abundance on the intestinal and hepatic tissues in human and other living organisms. CYPs enzymes are metabolizing a large variety of xenobiotic substances. Activities of cytochromes P450 enzymes are influenced by a variety of factors, such as genus, environmental, disease state, herbicide, alcohol, and herbal medications. However, diet seems to play a major role. The mechanisms of action of dietary chemicals, macro- and micronutrients on specific cytochrome P450 isozymes have been extensively studied. Cytochromes show a genetic polymorphism intra- or inter individual and intra- or interethnic. Moreover, effects of dietary modulation of the xenobiotic metabolism on chemical toxicity and carcinogenicity are stated. Bacteria have shown to have CYP-like genes. The tremendous metabolic capacity of the intestinal microbiota is associated to its enormous pool of CYP enzymes, which catalyzes reactions in phase I and phase II drug metabolism. Disease states, intestinal disturbances, ageing, environmental toxic effects, chemical exposures, or nutrition modulate the microbial metabolism of a drug before absorption.

A plethora of effects for most P 450, whereas concerns suppression or induction, can be resembled to pro-inflammatory cytokines and IFNs.

There are both pro-inflammatory cytokines and anti-inflammatory cytokines. Moreover, they are involved in the initiation and persistence of pathologic pain by directly activating sensory neurons and inflammatory cytokines are as well involved in different inflammation processes.

THE MICROBIOTA IN A MODEL OF ALZHEIMER'S DISEASE, AGING, AND DIETARY INTERVENTION

Cox, L.M.;*1.2 Schafer, M.J.;3.4 Sohn, J.;2 Weiner, H.L.;1 Ginsberg, S.D.;3.5 Blaser, M.J.^{2,6}

¹Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA USA

²Departments of Medicine and Microbiology, NYU Langone Medical Center, New York, NY USA

³Center for Dementia Research, Nathan Kline Institute, Orangeburg, NY USA ⁴Department of Cell Biology, NYU Langone Medical Center, New York, NY USA

⁵Departments of Psychiatry and Neuroscience & Physiology, NYU Langone Medical Center, New York, NY USA

⁶New York Harbor Department of Veterans Affairs Medical Center, New York, NY USA

Alzheimer's disease (AD), a progressive late-onset neurodegenerative disorder, is associated with accumulation of β-amyloid in senile plaques and tau in neurofibrillary tangles. AD pathology can be influenced by changes in diet, metabolism, and immunity, indicating that factors distant from the brain play a role in pathogenesis. The intestinal microbiota, composed of trillions of microbial cells, is hypothesized to influence AD pathobiology, but it's role remains to be adequately tested. We sought to determine whether there were specific microbial signatures associated with calorie restriction (CR)-mediated protection against accumulating AD pathology in the wellestablished mouse model of cerebral amyloid overexpression, the Tg2576 mouse. Tg2576 or nontransgenic (ntg) littermates were randomized to ad libitum (AL) or CR dietary regimens at ~3 months of age. CR significantly reduced amyloid plaque burden in Tg2576 mice, particularly in females. Fecal microbiota samples were collected over a 12-month timecourse, and microbial communities were surveyed by sequencing the 16S rRNA gene V4 region for 844 samples with a total depth of 13,386,491 reads. Tg2576 microbiota showed a trend of decreased phylogenetic diversity compared to ntg littermates, and CR showed a trend toward increased diversity compared to the AL diet. Several taxa were significantly altered by genotype, diet, or sex at multiple timepoints, which may be involved in either accelerating or preventing plaque pathology. This study characterizes key taxonomic changes and identifies particular intestinal microbiota as modifiable therapeutic targets in this murine model of AD. Further investigation of the interaction between the intestinal microbiota and agerelated neurodegenerative disorders in animal model systems is warranted to provide mechanistic insight into the development and potential abrogation of AD pathology.

THE FECAL MICROBIOME OF DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY

Isaiah, A.;* Parambeth, J.C.; Steiner, J.M.; Suchodolski, J.S. Gastrointestinal Laboratory, Texas A&M University, College Station, TX USA

Exocrine pancreatic insufficiency (EPI) in dogs is caused by the inadequate synthesis and secretion of pancreatic enzymes, which leads to clinical signs of maldigestion and malabsorption of nutrients. Previous studies using bacterial culture reported intestinal bacterial dysbiosis in dogs with EPI. However, there are no molecular studies evaluating the microbiome of dogs with EPI.

Objective. The objective of this study was to compare the fecal microbiome and predicted metagenome of healthy dogs (n=18), untreated (n=7) dogs with EPI, and dogs with EPI treated with enzyme replacement therapy (n=19).

Methods. To be included into the study, the dogs had to be at least 1 year of age, have clinical signs of EPI, a serum cTLI concentration \leq 2.5 µg/L, and be free from any other concurrent disease. Three naturally voided fecal samples collected over three consecutive days were frozen immediately after collection and pooled. Fecal samples were collected in a similar manner from healthy dogs without any clinical signs of gastrointestinal disease. Extracted DNA from fecal samples was used for Illumina sequencing of the bacterial 16S rRNA gene and analyzed using Quantitative Insights Into Microbial Ecology (QIIME). The software PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was used to predict functional gene content based on 16S rRNA gene data present in the Greengenes database and the KEGG database. Linear discriminant analysis (LDA) effect size (LEfSe) was performed to identify functional categories of statistical significance with the threshold of the logarithmic LDA score for discriminative features set at ≥2.5.

Results. There was a significant difference in fecal microbial communities when healthy dogs were compared to treated (p=0.001) and untreated (p=0.001) dogs with EPI. Alpha diversity was significantly decreased in untreated and treated EPI dogs when compared to the healthy dogs (p<0.01). The families Bifidobacteriaceae (p=0.006), Enterococcaceae (p=0.035), and Lactobacillaceae (p=0.001) were significantly increased in the untreated and treated dogs with EPI when compared to healthy dogs. Based on LEfSe, dogs with EPI (before treatment) had significant increases in functional genes associated with secretion systems, ABC transporters, fatty acid metabolism, Phosphotransferase system. In contrast, healthy dogs had a significant increase in genes related to Phenylalanine, tyrosine and tryptophan biosynthesis, and transcription machinery.

Conclusions. In conclusion, this study suggests that the fecal microbiome of dogs with EPI (both treated and untreated) is different from that of healthy dogs. The results also suggest that changes in bacterial groups are associated with functional alterations in the canine fecal microbiome and warrants further investigations

ESTABLISHMENT AND DEVELOPMENT OF INTESTINAL MICROBIOTA IN PRETERM INFANTS OF A LEBANESE TERTIARY HOSPITAL

Itani, T.;*1,3 Ayoub Moubareck, C.;^{1,3,6} Melki, I.;⁴ Delannoy, J.;² Mangin, I.;⁵ Butel, M.J.;² Karam Sarkis, D.^{1,3}

¹Microbiology Laboratory, School of Pharmacy, Saint-Joseph University, Beirut, Lebanon

²EA4065, Université Paris Descartes, Paris, France

³Rodolphe Mérieux Laboratory, Beirut, Lebanon

⁴Hôtel Dieu de France Hospital, Beirut, Lebanon

⁵Laboratoire MIEL, Cnam, Paris, France

⁶Department of Natural Science and Public Health, College of Sustainability Sciences and Humanities, Zayed University, Dubai, United Arab Emirates

The establishment and development of intestinal microbiota have been associated to profound, short or long term influences on health of full-term infants (FT) and preterm infants (PT). While some interesting studies exist for FT worldwide, little information is available for PI and no relevant data exist for the Lebanese population.

In this study, the composition and dynamics of the intestinal microbiota was determined during the first month of life for PT (n=66) and FT (n=17). Fecal samples were collected weekly and analyzed by molecular techniques, i.e. q-PCR and TTGE. Differences in the intestinal microbiota composition and establishment were observed between both groups. q-PCR showed that PT were more colonized by Staphylococcus than FT at weeks 1, 2, and 3; while FT were more colonized by Clostridium clusters I and XI. At one month of life, PT were mainly colonized by facultative anaerobes and some strict anaerobes like Clostridium cluster I and Bifidobacterium, while showing a delay in colonization by Bacteroides, Prevotella, Clostridium cluster XI, Clostridium leptum group, and Clostridium coccoides group. Types of feeding and antibiotic (AB) treatments had significant effects on intestinal colonization as Clostridium leptum group colonized more formula-fed and non-AB treated PT, while Bifidobacterium colonized less treated PT. TTGE analysis revealed low species diversity in both FT and PT. Inter-individual variability is higher in PT samples than in FT ones, which have quite similar patterns.

Our findings show that PT have an altered intestinal bacterial colonization compared with FT infants as previously described in other countries. This indicates the need for intervention strategies to mitigate the influence of intestinal microbiota on global health.

CHARACTERIZATION OF PHENOTYPIC AND GENETIC DIVERSITY AMONG LACHNOSPIRACEAE ISOLATES FROM THE HUMAN GUT MICROBIOTA

Lau, J.T.;* Surette, M.G. McMaster University, Hamilton, ON Canada

The Lachnospiraceae family, which is part of the Clostridiales order, is a prevalent and abundant member of the human gut microbiota, comprising up to 50% of the community. There is extensive diversity within the Lachnospiraceae; however, they are poorly studied, likely because they are strict obligate anaerobes and difficult to cultivate. We have explored the diversity of this family through phenotypic assays and whole genome sequencing of Lachnospiraceae strains isolated from human fecal samples. Using a method of culture-enriched molecular profiling, we previously determined that Lachnospiraceae could be enriched on anaerobic Cooked Meat agar and Brain Heart Infusion agar supplemented with inulin. Two fecal samples were cultured on these media, and 79 Lachnospiraceae isolates were obtained (>50% of the colonies sequenced were Lachnospiraceae), representing 32 different species. Isolates were characterized for biofilm production, auxotrophy, antibiotic resistance, motility, hemolysis, and exoenzyme production—which are important in bacterial-host interactions. Extensive phenotypic diversity was observed among Lachnospiraceae isolates of different species, but also among isolates of the same species that were isolated from the same individual. Comparison of the 16S rRNA gene sequence to reference isolates from the Ribosomal Database Project revealed 18 novel species. Additionally, 12 isolates were part of the Human Microbiome Project's Most Wanted taxa. Whole genome sequencing of a subset of isolates allowed for comparative genomic analysis, and preliminary data suggests that extensive genetic novelty is present in these isolates that is not currently represented in reference genomes. In order to fully appreciate the biological role and functional potential of the Lachnospiraceae in human gut microbiota, it is important to fully characterize the phenotypic and genotypic diversity in this family.

GUT MICROBIOTA IN HEALTHY SUBJECTS AND INPATIENTS WITH CLOSTRIDIUM DIFFICILE INFECTION

Flecher, T.B.; Miranda, K.R.; 2 Secco, D.A.; Peixoto, R.S.; Rosado, A.S.; do Carmo, F.L.; de Jesus, H.E.; Antunes, L.C.M., de Paula, G.R.; Domingues, R.M.C.P.

¹Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

²Faculdade de Farmácia, Universidade Federal do Rio de Janeiro – Campus Macaé, Macaé, RJ, Brazil

³Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

⁴Departamento de Tecnologia Farmacêutica, Universidade Federal Fluminense, Niterói, RJ, Brazil

The intestinal microbiota is a community of microorganisms that reside in the gastrointestinal tract without causing any harm to its host. Such microbial community forms a complex network of interactions, where symbiotic relationships that contribute to human health occur. Clostridium difficile is a nosocomial enteric pathogen, considered the main etiological agent of antibiotic-associated diarrhea in hospitals. C. difficile presents resistance to several antimicrobial agents used in empiric treatment protocols, which confers selective advantages to this species in comparison to others members of the intestinal microbiota. The aim of this study was to evaluate and identify possible alterations of the intestinal microbiota population resulting from the use of antimicrobials associated with infections by *C. difficile* (CDI). DGGE was used to evaluate fecal samples from two groups: healthy subjects and patients with C. difficile-associated diarrhea (CDAD). A decrease in bacterial diversity was highlighted by the low number of bands in samples from CDAD patients, compared to healthy subjects. This may indicate that antibiotic treatment would affect bacterial diversity, leading to a significant difference between the intestinal microbiota of these two groups of individuals, but further studies are still needed. Firmicutes and Verrucomicrobia phyla were detected mainly in healthy individuals, and these could be related to protection factors against the CDI. Ruminococcus bromii and Klebsiella variicola/K. pneumoniae were found mostly in samples from CDI patients. This study shows the effects of antimicrobials and the CDI itself on human intestinal microbiota.

Financial support: CAPES/FAPERJ, CNPq, CAPES, FAPERJ

EFFECT OF TOPSOIL EXPOSURE DURING LACTATION ON SUBSEQUENT PERFORMANCE AND GUT MICROBIOTA IN PIGS

Vo, N.;*1 Tsai, T.;² Kim, H.;² Sales, M.A.;² Wang, X.;² Erf, G.E.,³. Kegley, E.B.;² van der Merwe, M.,⁴ Buddington, R.,⁴ Maxwell, C.V.;² Carbonero, F.¹.⁵ ¹Cellular and Microbiology Program; University of Arkansas, Fayetteville AR USA

²Department of Animal Science; University of Arkansas, Fayetteville, AR USA ³Department of Poultry Science; University of Arkansas, Fayetteville, AR USA ⁴University of Memphis, Memphis, TN USA

⁵Department of Food Science; University of Arkansas, Fayetteville, AR USA

Sows (PIC-29) with litter size >10 (n=20) were blocked by BW and parity and assigned to litters either managed conventionally in farrowing crates (C) or exposed to topsoil (S) from d 4 postpartum to end of lactation. At weaning, five unisex littermates were penned together. Pigs were fed common antibiotic-free corn-SBM-DDGS nutrient-adequate diets. Intake and BW were recorded by phase throughout the nursery and grow-finish periods to determine ADG, ADFI, and G:F. Gut microbiota was determined from fecal microbial DNA by Illumina sequencing of the 16S rRNA gene sequence. Despite lower weaning BW (6.87 vs. 7.40 kg, P < 0.05), S pigs had higher greater ADG throughout the nursery period (0.42 vs. 0.35 kg/d, P < 0.01), resulting in S pigs being 2.21 kg heavier (P < 0.05) S than C pigs at the end of the nursery period. At study completion, S pigs tended (P > 0.10) to be 4.6 kg heavier than C pigs. Moreover, S pigs had higher greater ADFI than C pigs for overall nursery (0.62 vs. 0.51 kg/d; P < 0.01) and grow-finish periods (2.90 vs. 2.74 kg/d; P < 0.05). Although S pigs had higher greater G:F at nursery phases 1-2 (0.68 vs. 0.58; P = 0.02), they had lower G:F than C pigs at overall grow-finish (0.36 vs. 0.38; P < 0.05). Right after weaning, very significant changes in gut microbiota composition were observed in S pigs. Most notably, Abundances of *Clostridium* cluster XI and XIV, Faecalibacterium, Blautia, Butyriciococcus, and Lactobacillaceae were higher while Campylobacter and Bilophila were lower. Those trends tended to fade during the pigs development, where the main change was from a microbiota dominated by Bacteroides to one dominated by Prevotella. These results suggest that early exposure to dirt may benefit the gut microbiota and pigs general health, but that changes in the gut microbiota are not persistent. It is likely that early exposure to a diversity of microbes is instrumental in the immune system education and other factors that will then directly impact health, as postulated by the Hygiene Hypothesis.

1315	POSTER SESSION I: STUDENT PRESENTATION POSTERS	,
SP-1	The Enzymes of <i>Fusobacterium</i> spp. Involved in Hydrogen Sulfide Production from L-Cysteine	126
	Basic, A.;* Blomqvist, M.; Dahlén, G.; Svensäter, G.	
SP-2	Re-examining the Germination Phenotypes of Several <i>C. difficile</i> Strains	127
	Bhattacharjee, D.;* Francis, M.B.; Ding, X.; McAllister, K.M.; Shrestha, R.; Sorg, J.A.	
SP-3	Relationship Between Host Innate Immune Genetic Variation, Bacterial Vaginosis, Bacterial Colonization, and Cervicovaginal Cytokine Concentrations	128
	dela Cruz, E.J.;* Hawn, T.R.; Wiser, A.; Fredricks, D.N.; Marrazzo, J.M.	
SP-4	Clostridium difficile Colonizes Alternative Nutrient Niches Across Susceptible Gut Community Structures	129
	Jenior, M.L.;* Leslie, J.L.; Schloss, P.D.	
SP-5	Characterization of a Candidate Heme Detoxicification Operon in Clostridium difficile	130
	Knippel, R.J.;* Zackular, J.P.; Moore, J.L.; Skaar, E.P.	
SP-6	Collagen-Like Protein BclA3 in <i>Clostridium difficile</i> Spores Influence the Adherence to Epithelial Caco-2 Cells	131
	Pizarro-Guajardo, M.;* Brito-Silva, C.; Kuehne, S.A.; Minton, N.P.; Paredes-Sabja, D.	
SP-7	Binding of Clostridium difficile to Extracelular Matrix Proteins	132
	Santos, M.G.C.;* Trindade, C.N.R.; Rocha-Azevedo, B.; Ferreira, E.O.; Domingues, R.M.C.P.	
SP-8	Characterization of Clinically Relevant Genetically Tractable Clostridium difficile Strain R20291 in a Mouse Model	133
	Winston, J.A.;* Thanissery, R.S.; Montgomery, S.A.; Theriot, C.M.	
SP-9	The Potential of Using Metabonomic Approaches to Studying the Human Gut Microbiome	134
	Yen, S.;* Aucoin, M.G.; Allen-Vercoe, E.	

Posters will be presented in Poster Session I Tuesday, July 12 1315-1415.

THE ENZYMES OF FUSOBACTERIUM SPP. INVOLVED IN HYDROGEN SULFIDE PRODUCTION FROM L-CYSTEINE

Basic, A.;*1 Blomqvist, M.;2 Dahlén, G.;1 Svensäter, G.²
¹Oral Microbiology and Immunology Institute of Odontolog

¹Oral Microbiology and Immunology, Institute of Odontology, University of Gothenburg, Gothenburg, Sweden

²Oral Biology, Institute of Odontology, Malmö University, Malmö, Sweden

The proteins of *Fusobacterium* spp. were studied with regard to their ability to produce hydrogen sulfide (H₂S) from L-cysteine. H₂S is a toxic foulsmelling gas produced by subgingival biofilms in patients with periodontal disease (toothloss) and is suggested to be part of the pathogenesis of the disease. Proteins extracted from different Fusobacterium spp. were screened with 1D gel electrophoresis for their H₂S producing ability and detected enzymes were also subjected to a 2D gel electrophoresis with the same in-gel activity assay test; Sulfide from H₂S, produced by the enzymes in the gel, reacted with bismuth forming bismutsulfide, illustrated as brown bands (1D) or spots (2D) in the gel. The enzymes from 2D gel electrophoresis were extracted and identified with liquid chromatography—tandem mass spectrometry (LC-MS/MS). Cysteine synthase and proteins involved in the production of the coenzyme pyridoxal 5' phosphate (that catalyzes the production of H₂S) were frequently found among the identified enzymes. Interestingly, a higher expression of H₂S producing enzymes was detected from bacteria incubated without cysteine prior to the experiment. This study screened for proteins with regard to H₂S production from L-cysteine, confirmed the high capacity of H₂S production from Fusobacterium spp. but also the variance in enzymatic pathways among different spp. and strains. Cysteine synthase was frequently identified among the extracted proteins from different Fusobacterium spp. involved in the production of H₂S. The reason for the higher H₂S producing proteome expression among bacteria incubated in broth without cysteine is unknown but it may be that there is a limit for bacterial production of H₂S before it becomes toxic for the bacteria, as well. Knowledge of the mechanisms involved in the production of H₂S creates a platform for future detection and evaluation of its role in the pathogenesis of periodontal disease. Further, knowledge of the enzymatic activity may be used to manipulate the production of H₂S as a possible treatment strategy.

RE-EXAMINING THE GERMINATION PHENOTYPES OF SEVERAL C. DIFFICILE STRAINS

Bhattacharjee, D.;* Francis, M.B.; Ding, X.; McAllister, K.M.; Shrestha, R.; Sorg, J.A.

Department of Biology, Texas A&M University, College Station, TX USA

C. difficile spores survive for extended periods of time outside the host. Inside a host, the spores germinate to the vegetative forms, which produce toxins that elicit the primary symptoms of disease. Previous work by our lab, and others, has shown that *C. difficile* spores germinate *in vitro* in response to certain bile acids and glycine. Cholic acid-derivatives [e.g. taurocholic acid (TA)] initiate spore germination and chenodeoxycholic acid-derivatives (CDCA) act as competitive inhibitors of cholic acid-mediated germination. Recently, spores derived from some C. difficile clinical isolates were reported to germinate in rich medum without TA and/or germination was not be inhibited by CDCA. Because the mechanisms of *C. difficile* spore germination as beginning to be elucidated, the phenotypes associated with these strains could yield important information on *C. difficile* spore germination. Here, we further analyzed the reported clinical strains by quantifying interaction of the bile acids with the *C. difficile* spores, the abundance of DPA in the spore and levels of ther germinant receptor complex (the bile acid germinant receptor, CspC, the germination-specific protease, CspB, and the cortex hydrolase, SleC). Upon re-examining these strains, we determined that all strains tested had the requirement for bile acids to stimulate germination, are inhibited by CDCA, and exhibit variability in the abundance of the germinant receptor complex. Finally, by comparing the rates of germination in these strains to the abundance of CspC or to the ratio of CspC to SleC, we hypothesize that CspC acts to initiate spore germination but then inhibits downstream processes until another signal is received.

RELATIONSHIP BETWEEN HOST INNATE IMMUNE GENETIC VARIATION, BACTERIAL VAGINOSIS, BACTERIAL COLONIZATION, AND CERVICOVAGINAL CYTOKINE CONCENTRATIONS

dela Cruz, E.J.;*^{1,2} Hawn, T.R.;² Wiser, A.;¹ Fredricks, D.N.;^{1,2} Marrazzo, J.M.³ ¹University of Washington, Seattle, WA USA ²Fred Hutchinson Cancer Research Center, Seattle, WA USA ³University of Alabama, Birmingham AL USA

Purpose: Our goals were to identify host innate immune genotypes associated with prevalent bacterial vaginosis (BV) and colonization with BV-associated bacteria (BVAB), and to characterize the effect of BVAB colonization and host genotype on pro-inflammatory cytokines in the cervicovaginal milieu.

Methods: We enrolled women with and without BV in a study of the vaginal microbiota. Human genomic DNA was extracted from whole blood collected from study participants at enrollment and used to genotype a panel of 14 single nucleotide polymorphisms (SNPs) in toll-like receptor-1 (TLR1), TLR2, TLR4, TLR5, as well as regulators of TLR signaling, toll-interacting protein (TOLLIP) and CD180. Colonization with 15 bacterial species was assessed by quantitative PCR assays applied to genomic DNA extracted from vaginal swabs. Cervicovaginal lavage fluid (CVL) from a subsample of participants was used to measure interleukin-6 (IL-6), IL-8, and IL-1b using the Luminex platform.

Results: În an analysis restricted to n=44 non-Hispanic, Caucasian participants, we found a relationship between TLR1 deficiency (defined by genotype TLR1 1805GG) and decreased probability of prevalent BV (p=0.005) as well as colonization with BVAB1 (p=0.013), *Megasphaera* genogroups 1 and 2 (p=0.008), and *Prevotella annii* (p=0.004). Homozygotes for the minor allele of TOLLIP_rs3750920 had decreased probability of *Gardnerella vaginalis* colonization (p=0.020), while heterozygotes for TOLLIP_rs3793964 had decreased probability of *Mageebacillus indolicus* colonization (p=0.037). In an subsample of n=23 study participants of all race/ethnicity backgrounds, carriers and homozygotes for TLR1 deficiency trended towards increased concentrations of IL-6 and IL-1b in CVL.

Conclusions: We found correlations between TLR1 deficiency and BV status, colonization with BVAB, and cervicovaginal cytokine concentrations. These results suggest that variation in innate immunity may influence risk of BV and inflammatory response to BVABs.

CLOSTRIDIUM DIFFICILE COLONIZES ALTERNATIVE NUTRIENT NICHES ACROSS SUSCEPTIBLE GUT COMMUNITY STRUCTURES

Jenior, M.L.;* Leslie, J.L.; Schloss, P.D. University of Michigan, Ann Arbor, MI USA

Clostridium difficile infection has become the most common hospital-acquired bacterial infection and causes a toxin-mediated diarrheal disease. Intestinal colonization of *C. difficile* is dependent on a reduction in colonization resistance after perturbation of the indigenous gut microbiota. Previous studies from our lab have demonstrated different classes of antibiotics, each resulting in a unique bacterial community structure, still fail to resist colonization by *C. difficile* (~10⁸ CFU per gram cecal content). Presumably, nutrient availability would differ across each of these conditions, and C. difficile would need to adapt in order to acquire growth substrates. Currently, measuring metabolite flux at an individual organism level in a whole community is not feasible. With this in mind, I employed community-wide transcriptional analysis to measure the metabolic activity of both C. difficile during infection, as well as the remaining bacterial populations. Results from these experiments indicate *C. difficile* may alter its expression profile in response to variable community structure. These changes include an upregulaton of several glycan and amino acid scavenging pathways that inversely correlates with the diversity of the surrounding bacterial community. *C. difficile* toxin is also significantly more detectable (p < 0.005) in low diversity communities. Furthermore, metagenome-enabled metatranscriptomic analysis (~3.56 Gb transcripts mapping to ~341.40 Mb genes) reveals that transcripts associated with these pathways are underrepresented when compared to communities in untreated mice. Additionally, I apply genome-scale metabolic-modeling techniques to the transcriptome of *C. difficile*, in order to infer metabolite utilization hierarchy. Using these strategies, preliminary results indicate that the catabolism of specific mucus-derived monosaccharides tracks with the capacity for their cleavage by other bacteria. This technique will also be applied to metagenomes to infer community-level metabolism and niche segregation. By understanding how C. difficile may exploit susceptible gut communities, targeted therapies for preventing its colonization may be developed.

CHARACTERIZATION OF A CANDIDATE HEME DETOXICIFICATION OPERON IN CLOSTRIDIUM DIFFICILE

Knippel, R.J.;*1 Zackular, J.P.;1 Moore, J.L.;3 Skaar, E.P.1,2,4

¹Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN USA

²Vanderbilt Institute for Chemical Biology, Vanderbilt University, Nashville, TN USA

³Department of Chemistry, Vanderbilt University, Nashville, TN USA ⁴Tennessee Valley Healthcare System, U.S. Department of Veterans Affairs, Nashville, TN USA

Clostridium difficile is a Gram-positive, spore-forming human pathogen that infects the colon, causing a wide range of disease from infectious diarrhea to fulminant colitis. In the last decade, the number of *C. difficile* infections have dramatically risen. Surprisingly, the mechanisms by which C. difficile acquires nutrients during infection is largely unknown. During serious C. difficile infection, host hemoglobin becomes highly abundant in the gastrointestinal lumen. As the main source of iron within the host is sequestered in the heme of hemoglobin, this provides a rich metabolic reservoir. Paradoxically, high levels of heme can often be toxic to bacteria. Interestingly, the impact of heme on *C. difficile* has not been characterized. We hypothesized that *C. difficile* utilizes heme as a nutrient source and simultaneously detoxifies excess heme to survive within the lumen during infection. An RNA-seq analysis of C. difficile R20291 in the presence of a sub-toxic concentration of heme discovered the up-regulation of an operon, which contains a candidate TetR family transcriptional regulator, renamed heme activated regulator (harR), and a major facilitator superfamily transporter, renamed heme activated transporter (hatA). To elucidate the function of this operon, a genetic knockout of harR (ΔharR) was constructed using ClosTron technology and subjected to various heme toxicity and heme adaptation assays. These experiments revealed that ΔharR is more sensitive to heme and unable to adapt to heme toxicity when compared to the wildtype strain. Together these data suggest that harR and hatA play a critical role in resisting heme toxicity in the host. Understanding the mechanism of heme resistance could lead to novel therapeutic strategies to combat *C*. difficile.

COLLAGEN-LIKE PROTEIN BCLA3 IN *CLOSTRIDIUM* DIFFICILE SPORES INFLUENCE THE ADHERENCE TO EPITHELIAL CACO-2 CELLS

Pizarro-Guajardo, M.;*¹ Brito-Silva, C.;¹ Kuehne, S.A.;² Minton, N.P.;³ Paredes-Sabja, D.¹

¹Gut Microbiota and Clostridia Research Group, Department of Biological Sciences, Universidad Andrés Bello, Santiago, Chile

²Clostridia Research Group, Centre for Biomolecular Sciences, School of Life Sciences, University of Nottingham, Nottingham UK.

³Clostridia Research Group, BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), School of Life Sciences, University of Nottingham, Nottingham UK

Background & Aims: Collagen, like proteins, are present in the spore of several spore producing bacteria. In *Clostridium difficile*, most of the strains described exhibit collagen like proteins, called BclA1, BclA2, and BclA3, in the external layer of the spores: the exosporium. The exosporium of epidemic strains of *C. difficile* and other spore former bacteria exhibit exosporium-filaments, which in *B. cereus* group correspond to collagen like proteins and are implicated in adherence to the host. In this work, we want to evaluate the role of BclA3 in the spore and its adherence to Caco-2 cells.

Methods: By homology recombination, we constructed the mutant of BclA3 in R20291 (CD3193) and evaluate the resulting phenotype. In order to identify the composition of the filaments of the exosporium, we evaluate by transmission electron microscopy the mutant spores. To assess the influence of this protein in the anchorage and localization of BclA2 we used immunofluorescence and western blot with anti-BclA2 antibody. As exosporium is responsible for the spore hydrophobicity, we also measure the contribution of BclA3 to hydrophobicity by hexadecane assay. Finally, we evaluated if BclA3 affects the adherence to differentiated Caco-2 monolayer.

Results: BclA3 is part of the exosporium filaments as shown by TEM and affects localization of BclA2 in the spore, as seen by relative abundance of BclA2 in the exosporium, and the hydrophobicity is also altered by the mutation of this protein. All this characteristics of the mutant lead to the differences, we have found in the adherence to Caco-2 cells between wild type and mutant.

Conclusions: BclA3 as implicances on adherence to epithelial cells. BclA3 probably plays a competitive and redundant role with BclA2. *In vivo* studies are necessary to describe the possible role of BclA3 in pathogenesis.

BINDING OF *CLOSTRIDIUM DIFFICILE* TO EXTRACELULAR MATRIX PROTEINS

Santos, M.G.C.;*1 Trindade, C.N.R.;1 Rocha-Azevedo, B.;3 Ferreira, E.O.;1.2 Domingues, R.M.C.P.1

¹Laboratório de Biologia de anaeróbios, IMPG, Universidade Federal do Rio de Janeiro, Brazil

²UFRJ- Pólo Xerém, Rio de Janeiro, Brazil

³Laboratório de Cardiologia Celular e Molecular, IBCCF, UFRJ, Rio de Janeiro, Brazil

Clostridium difficile (Cdiff) is the most common cause of nosocomial diarrhea. Several adhesins have been reported and might contribute to Cdiff colonization and infection, but only few studies about Cdiff binding to extracellular matrix (ECM) components have been reported. Thus, the aim of this work is to study the binding of different Cdiff ribotypes (RT027, RT133, RT135, and RT012) to collagen (COL) type IV and V and laminin-1 (LMN-1). All strains were grown in BHI-PRAS in the absence or presence of different glucose (0.2%, 0.5% and 1%) concentrations. For binding quantification, 24-well plates with coverslips previously coated with COL IV and LMN-1 (20µg/mL) were prepared. Bacteria were added for 1h under anaerobic conditions at 37°C. Samples stained with a Live/dead bacterial kit (Invitrogen) were used for image acquistion and ImageJ was used for quantification. Data show a greater adhesion of the RT012 to LMN-1 and COL IV in all three glucose concentrations. For RT027 and RT135 strains the strongest adhesion was observed with 0.5% glucose. RT133 did adhere to neither LMN-1 nor COL IV, as previously shown. In the absence of glucose, the adhesion was poor in all ribotypes. Since adhesion was enhanced by glucose, a biofilm production test was conducted, showing that three ribotypes, except for RT027, are major biofilm producers. Experiments are carried on to determine what protein(s) are responsible for ECM binding. Mass spectrometry (MALDI TOF/TOF) will be used to identify proteins and data will be analyzed by MASCOT (NCBInr database). Our results show that Cdiff can recognize different ECM components, depending on their ribotypes and raise the possibility of the involvement of biofilm proteins in ECM binding. We believe protein identification can lead to potential protein targets among ribotypes and possible elucidation of Cdiff colonization and inflammatory disease.

Financial support: CAPES, FAPERJ, CNPq and "Ciências Sem Fronteiras" Program.

CHARACTERIZATION OF CLINICALLY RELEVANT GENETICALLY TRACTABLE *CLOSTRIDIUM DIFFICILE* STRAIN R20291 IN A MOUSE MODEL

Winston, J.A.;*¹ Thanissery, R.S.;¹ Montgomery, S.A.;² Theriot, C.M.¹

¹Department of Population Health and Pathobiology, NCSU, College of Veterinary Medicine, Raleigh, NC USA

²Pathology and Laboratory Medicine, UNC, School of Medicine, Chapel Hill, NC USA

Clostridium difficile infection (CDI) is associated with increasing morbidity and mortality, consequently posing an urgent threat to public health. Recurrence of CDI after successful treatment with antibiotics is high, occurring in 20-30% of patients, thus necessitating discovery of novel therapeutics against this pathogen. To evaluate therapeutics against C. difficile, a mouse model approximating human disease with a clinically relevant strain is needed. We aimed to characterize the clinical course, including weight loss, bacterial load, toxin activity, and histopathologic changes to the large intestine of mice challenged with clinically relevant genetically tractable C. difficile strain R20291. 5-8 week old C57BL/6 WT JAX mice were pretreated with cefoperazone in their drinking water (0.5 mg/ml) for 5 days, allowed a two-day wash out with regular water, and challenged with 10⁴ spores of C. difficile R20291 on day 0. Clinical signs of disease and alterations to the gut microbiota were monitored for a 14 day period. Mice were colonized with 10⁷ colony-forming units (CFU) of *C. difficile* per gram of feces on day 1. The most significant amount of weight loss and clinical signs of disease in mice were seen between day 2 and 5. Mice remained persistently colonized with C. difficile, however weight gain and resolution of clinical signs were observed after day 7. C. difficile toxin activity, intestinal histopathologic changes and alteration to the gut microbiota were defined throughout infection. Compared to other murine models of CDI, this model was not uniformly lethal at this dose allowing for observation of a prolonged clinical course of infection concordant with human disease. Overall, this mouse model proved a valuable experimental platform of CDI. Based on our findings, this fully characterized C. difficile murine model can be utilized to assess effects of novel therapeutics on amelioration of clinical disease and restoration of colonization resistance against CDI.

THE POTENTIAL OF USING METABONOMIC APPROACHES TO STUDYING THE HUMAN GUT MICROBIOME

Yen, S.;*1 Aucoin, M.G.;2 Allen-Vercoe, E.1

¹Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON Canada

²Waterloo Institute of Nanotechnology, Department of Chemical Engineering, University of Waterloo, Waterloo, ON Canada

Purpose: Here, we present the metabonomic approach that we have used to study the human gut ecosystem and highlight the characterizations that can be drawn from the metabonomic data.

Methods: Metabonomic study of the human gut microbiome offers a systemic evaluation of the interactions and metabolic processes occurring within the gut ecosystem. In growing gut microbiota as a community culture in an *in vitro* bioreactor system, we are able to study the metabolic interactions in a controlled manner, over an extended period of time. We have used nuclear magnetic resonance (NMR)-based metabonomics to describe several microbial consortia grown in vitro, each representing gut microbiota of different individuals.

Results: We have found that the metabolite profiles of these bacterial communities were unique to each ecosystem, yet at the same time, there were commonalities that seemed to reflect a set of core metabolic functions essential to these ecosystems. Amino acids were the most commonly found class of metabolites, but carboxylic acids (which include short chain and branch chain fatty acids) were the most abundant metabolites. This proportion of metabolite profiles was consistent to all the microbial ecosystems evaluated, indicating the carbohydrate fermentation is a conserved and dominant metabolism carried out by human gut microbiota. At the same time, metabolite patterns that are unique to a community highlight particular metabolic features that could be of interest.

Conclusion: This ability to make inferences on unique metabolic function, and the respective bacteria potentially responsible, is one of the strengths of metabonomic approaches. Metabonomic approaches have been used to identify biomarkers for diagnosis of gastrointestinal diseases, but can also be used to comment on the functional role of the gut microbiota in both dysbiotic and healthy states.

1300	SESSION II: ANAEROBES IN THE MOUTH	
PII-1	Oral Anaerobes of the Dorsum of the Tongue: An Experimental Study	136
	Mailharin, A.; Saint-Marc, M.; Badet, C.*	
PII-2	Implementation of Novel Species Identification into the Medical Microbiology Routine Diagnostics Workflow and Description of <i>Prevotella Colorescens</i> sp. nov. and <i>Prevotella Festinatalis</i> sp. nov. Buhl, M.;* Willmann, M.; Oberhettinger, P.; Liese, J.; Autenrieth, I.B.; Marschal, M.	137
PII-3	Predominant Bacterial Pathogens in Odontogenic Infections Egwari, L.O.;* Nwokoye, N.N.; Olubi, O.O.	138
PII-4	Three Variants of the Leukotoxin Gene in Human Isolates of Fusobacterium necrophorum Subspecies Funduliforme	139
	Holm, K.;* Collin, M.; Hagelskjaer-Kristensen, L.; Jensen, A.; Rasmussen, M.	
PII-5	Antimicrobial Susceptibilites of Infrequent <i>Prevotella</i> Strains from Saliva	140
	Könönen, E.;* Lehto, L.; Gürsoy, M.	
PII-6	Antimicrobial Susceptibilites of Salivary <i>Prevotella melaninogenica</i> , <i>Prevotella histicola</i> , and <i>Prevotella jejuni</i>	141
	Lehto, L.;* Gürsoy, M.; Könönen, E.	
PII-7	Recognition of Laminin by Pathogenic Oral <i>Prevotella</i> ssp. <i>Marre, A.T.O.;* Boente, R.F.; Ferreira, E.O.; Domingues, R.M.C.P.; Lobo, L.A.</i>	142
PII-8	Improvement of an Experimental Model of Oral Biofilm <i>Nguyen, D.;</i> * <i>Badet, C.</i>	143
PII-9	Functional Genomics in an Oral Campylobacter, Campylobacter rectus	144
	Threadgill, D.S.;* Conley, B.A.; Harrell, E.A.	
PII-10	Detection of Fusobacterium necrophorum from Pediatric Patient Throat Cultures by Selective Anaerobic Medium Very T.T.* Core M. F. Core M. F. Direct Paral I.	145
	Van, T.T.;* Cox, L.M.; Cox, M.E.; Dien Bard, J.	

Posters will be presented in Poster Session II Wednesday, July 13 1300-1400.

ORAL ANAEROBES OF THE DORSUM OF THE TONGUE: AN EXPERIMENTAL STUDY

Mailharin, A.;¹ Saint-Marc, M.;¹ Badet C.*¹.².
¹Univ. de Bordeaux, Cours de la Marne 33082 Bordeaux, France
²Univ. Bordeaux, ISVV, EA 4577 Unité de recherche Oenologie, Institut Polytechnique de Bordeaux, F-33140 Villenave d'Ornon, France

The dorsal surface of the tongue, because of its papillary structure, is highly colonized by bacteria and, thus could play a crucial role in the emergence, or the recurrence of oral diseases. The aim of our work was to compare the anaerobes present within the lingual flora between patients with periodontitis and a control group. Twenty adults suffering from periodontitis and twenty healthy adults participated in the study. All subjects were examined for dental caries, dental plaque, and calculus accumulation. They were asked for their oral hygiene habits and for their bad breath feeling. The organoleptic assessement of halitosis was also carried out by the investigator. Samplings were done by gently scraping the dorsal tongue surface with a sterilized toothbrush. After dispersing, the samples were diluted and spiral plated on two media, Fastidious Anaerobe Agar and Columbia Blood agar. Plates were incubated at 37°C. in an anaerobic jar for 2-4 days. Each different colony was observed, counted and Gram stained. Identifications were carried out using both biochemical (api 20A) and PCR methods.

The Student test was used to compare the average of Log CFU on the different culture media among the two studied groups. The data were analysed with GraphPad. We considered P values of < 0.5 to be signficant. Concerning the number of colonies grown on the two media, no statistical differences were observed either between the control group and the subjects with periodontitis or between the two groups concerning halitosis. Concerning the qualitative analysis, one hundred and nine strains have been identified and six remain unknown. The identified bacteria belong to seventeen different species.

The periodontal pathogens represent half of all species found in the lingual flora of patients suffering from periodontitis, against 30% in the control group. This result suggests that the lingual flora could play a role in re-infection of the sub-gingival plaque after periodontal treatment.

IMPLEMENTATION OF NOVEL SPECIES IDENTIFICATION INTO THE MEDICAL MICROBIOLOGY ROUTINE DIAGNOSTICS WORKFLOW AND DESCRIPTION OF *PREVOTELLA COLORESCENS* SP. NOV. AND *PREVOTELLA FESTINATALIS* SP. NOV.

Buhl, M.;* Willmann, M.; Oberhettinger, P.; Liese, J.; Autenrieth, I.B.; Marschal, M.

University Hospital Tübingen, Institute of Medical Microbiology and Hygiene, Tübingen, Germany

The current study describes the characterization of anaerobes within the medical microbiology routine diagnostics workflow, resulting in the description of two novel *Prevotella* species.

Prevotella sp. are obligate anaerobes and form part of the human oral and gastrointestinal microbiota, but may cause severe infections if translocated to other body sites. In the medical microbiology context, correct identification of anaerobes has been facilitated in recent years by the introduction of mass spectroscopy and gene sequencing, which are of great diagnostic advantage to the hitherto phenotypically based methods. We have implemented a routine diagnostics workflow, allowing the identification of anaerobes to the species level by using MALDI-TOF-MS with a constantly growing database of spectra. Three strains which could initially not be identified on the species level, but as belonging to the genus *Prevotella*, were investigated further and proved to be novel species.

The three strains (A1336, A1722, and A2270) appeared as Gramnegative, and electron microscopy revealed rod-shaped bacteria with some intercellular matrix adhesion. All three strains were moderately proteolytic and saccharolytic, with strains A1722 and A2270 exhibiting the same characteristics (API rapid ID 32A, sugar fermentation in peptone-yeast extract broth). The G+C content of the DNA was 43.2 (A1336) and 43.6 mol% (A1722), respectively, as determined by GC and whole-genome-sequencing. Phylogenetic analysis was based on full-length 16S rRNA gene sequence and showed the three strains to belong to the genus *Prevotella*, but to be different from the other *Prevotella* species and from *Hallella seregens* (closest sequence similarity phylogenetic trees were constructed by the maximum likelihood model).

In the view of phenotypic and biochemical properties as well as gene sequencing, the three strains are considered to belong to two novel species within the genus *Prevotella*, with the proposed names of *Prevotella colorescens* sp. nov. (strain A1336) and *Prevotella festinatalis* sp. nov. (strains A1722 and A2270).

PREDOMINANT BACTERIAL PATHOGENS IN ODONTOGENIC INFECTIONS

Egwari, L.O.;*1 Nwokoye, N.N.;2 Olubi, O.O.3

*Department of Biological Sciences, College of Science and Technology, Covenant University, Canaanland, Ota, Ogun State, Nigeria

²National Tuberculosis Reference Laboratory, Microbiology Division, Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria

³Department of Ear, Nose and Throat, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

Odontogenic infections originate in either the dental pulp or the periodontium. The diversity of microorganisms in the oral cavity and the ingress of microbes from the external make it essential to define the etiologies of odontogenic infections and establish possible microbial association in the disease process. Four clinical conditions were studied; dentoalveolar abscess, gingivitis, periodontitis, and odontogenic tumors (osteosarcoma and fibrosarcoma). Odontogenic infections including the tumors were characterized by polymicrobial etiologies. Aerobes were the dominant flora in odontogenic tumors and gingivitis (Streptococcus, Enterococcus, Staphylococcus, Pseudomonas, Proteus, Klebsiella, and Escherichia) while anaerobes predominated in dentoalveolar abscess and periodontitis (Porphyromonas, Prevotella, Fusobacterium, and Aggregatibacter). Monoetiological status was obtained for S. pyogenes and S. aureus in two cases of gingivitis and α -hemolytic streptococci in a case of gingivitis. Porphyromonas gingivalis was isolated in pure cultures from two cases of periodontitis. Aggregatibacter actinomycetemcomitans was isolated from 6 (6.4%) cases of periodontitis but was not a component of the gingival flora. The peptostreptococci were isolated more from cases of periodontitis (11.7%) than from gingivitis (8.8%). The most frequent association observed was those between A. actinomycetemcomitans and Fusobacterium nucleatum, Porphyromonas spp. and Prevotella spp. and association between Peptostreptococcus, Prevotella and facultative anaerobes (staphylococci and escherichia). Polymicrobial etiology was imperative in odontogenic diseases; a shift of aerobic dominance in gingivitis and odontogenic tumors to anaerobic preponderance in dentoalveolar abscess and periodontitis.

THREE VARIANTS OF THE LEUKOTOXIN GENE IN HUMAN ISOLATES OF FUSOBACTERIUM NECROPHORUM SUBSPECIES FUNDULIFORME

Holm, K.;*1 Collin, M.;¹ Hagelskjaer-Kristensen, L.;² Jensen, A.;³ Rasmussen, M.¹

¹Department of Clinical Sciences, Division of Infection Medicine, Lund University, Lund, Sweden

²Department of Clinical Microbiology, Viborg Hospital, Viborg, Denmark ³Department of Biomedicine, Faculty of Health Sciences, Aarhus University, Aarhus, Denmark

Purpose: Leukotoxin is a well known virulence factor of animal isolates of *Fusobacterium necrophorum* subspecies *necrophorum*, and is also expressed by animal isolates of subspecies *funduliforme*, whereas its presence in human isolates has not been fully elucidated. The aims of this study were to determine the presence of the leukotoxin gene in human isolates of *F. necrophorum* subspecies *funduliforme*, to determine the nucleotide sequence, and to determine if there are sequence differences between isolates from different clinical conditions.

Methods and results: The leukotoxin gene from ten invasive isolates was sequenced and found to be of three different sequence types: Two isolates had a leukotoxin sequence identical to the previously published leukotoxin sequence from a human isolate (lkt type 1a), four isolates had a two nucleotide deletion resulting in a putative truncated protein with a molecular mass of abut 140 kDa instead of 337 kDa (lkt type 1b), and four isolates had a distinctly different sequence with only 78% nucleotide identity to the previously published sequence (lkt type 2). Next, a total of 221 isolates were examined by PCR using primers specific for the type 1 or type 2 sequence flanking the region of the lkt type 1b deletion, followed by sequencing of the *type 1* PCR products. The leukotoxin gene was present in all isolates studied. 137 of the isolates belonged to type 2, 55 to type 1b and only 29 isolates to the previously described type 1a sequence. The type 1b sequence was significantly more common than type 1a among isolates derived from head-and-neck infections compared with isolates from non head-and-neck (mainly gastro-intestinal and urogenital) infections (Pairwise comparison, Fisher's exact test p = 0.027 after Bonferroni correction). The proportion of the *type 2* to *type 1* sequence did not differ significantly between the groups.

Conclusion: The leukotoxin gene was present in all human isolates of *F. necrophorum* tested, and three variants were found, two of which were not previously described. The sequence types seem to correlate to source of infection. Further studies are needed to elucidate the role of the leukotoxin in human infections.

ANTIMICROBIAL SUSCEPTIBILITES OF INFREQUENT PREVOTELLA STRAINS FROM SALIVA

Könönen, E.;* Lehto, L.; Gürsoy, M. Department of Periodontology, Institute of Dentistry, University of Turku, Turku, Finland

Objective: Anaerobic *Prevotella* organisms belong to the resident microbiota of the mouth. During the past years, several novel *Prevotella* species from various clinical sources have been described; however, information on their antimicrobial susceptibilities is mainly lacking. Here we present the antimicrobial susceptibility test results of 22 salivary *Prevotella* or *Alloprevotella* strains to nine antibiotics.

Material and Methods: The salivary strains included 3 *P. nanceiensis*, 10 *P. salivae*, 2 *P. scopos*, 1 *P. shahii*, 3 *Alloprevotella rava*, and 3 unnamed strains (2 *Prevotella* sp. oral taxon 313 and 1 *Prevotella* sp. oral taxon 317). Their identification was based on partial 16S rRNA sequencing (an identity score of 97% for genus and 99% for species level). The agar dilution method was used to test their minimal inhibitory concentrations (MICs) to amoxicillin (AMX), amoxicillin/clavulanate (AMC), azithromycin (AZM), ertapenem (ETP), cefoxitin (FOX), clindamycin (CLI), metronidazole (MTZ), moxifloxacin (MXF), and tetracycline (TET). The EUCAST and CLSI breakpoints, if available for anaerobes, were used to categorize the strains as susceptible, intermediate, or resistant to tested antibiotics.

Results: All 22 strains tested were susceptible to AZM, ETP, FOX, and CLI, whereas 9 (41%) were intermediate/resistant to TET, 6 (27%) to MXF, 3 (14%) to AMX, 2 (9%) to MTZ, and 1 (5%) to AMC. Of the 2 strains with increased MIC values to MTZ, *P. salivae* had MIC of 128 mg/L and *Prevotella* sp. oral taxon 313 16 mg/L. In addition to the MTZ-resistant *P. salivae* strain, 3 *P. salivae* strains had increased MIC values to TET, and 2 strains to AMC or MFX. Of the 3 *P. nanceiensis* strains, all were resistant to MFX, 2 to TET, and 1 multi-resistant strain showed also resistance to AMX and intermediate resistance to AMC. Furthermore, 1 *P. scopos* strain was resistant to AMX and intermediate to TET, 1 *P. shahii* strain was resistant to MXF, while all 3 *A. rava* strains were resistant to TET.

Conclusion: *P. nanceiensis*, originally isolated from infections outside the oral cavity, proved to be less susceptible than other *Prevotella* species tested. Two *Prevotella* strains were resistant to MTZ.

ANTIMICROBIAL SUSCEPTIBILITES OF SALIVARY PREVOTELLA MELANINOGENICA, PREVOTELLA HISTICOLA, AND PREVOTELLA JEJUNI

Lehto, L.;* Gürsoy, M.; Könönen, E. Department of Periodontology, Institute of Dentistry, University of Turku, Turku, Finland

Objective: *Prevotella melaninogenica* is known as a ubiquitous colonizer of the oral cavity and a frequent producer of β -lactamase. However, among closely related novel *Prevotella histicola* and *Prevotella jejuni*, data are still lacking. Interestingly, to date *P. jejuni* has been reported from a small intestine of a child only. In this study, β -lactamase production and *in vitro* resistance of 144 isolates representing the three species were tested to six antibiotics.

Material and Methods: Salivary isolates were collected from 37 post-partum women in 1994-1995, and later identified to the species level by partial 16S rRNA sequencing: *P. melaninogenica* (n=99), *P. histicola* (n=20), and *P. jejuni* (n=25). β-lactamase production was performed by the nitrocefin disk test. Minimal Inhibitory Concentrations (MICs) of amoxicillin (AMX), amoxicillin/clavulanate (AMC), ertapenem (ETP), cefoxitin (FOX), clindamycin (CLI), and metronidazole (MTZ) were determined using the agar dilution method. Resistance rates were based on the breakpoints provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI).

Results: Of the 144 isolates, 99 (69%) produced β-lactamase. The majority of P. jejuni (80%) and P. melaninogenica (73%) strains were positive for β-lactamase production, while 35% of P. histicola strains were positive. MIC (mg/L) of P. histicola to AMX ranged between <0.125-2 and to AMC <0.125-2. Corresponding ranges of P. jejuni were <0.125-2 and <0.125-16, and those of P. melaninogenica <0.125-128 and <0.125-32, respectively. According to CLSI breakpoints, the resistance rate of strains to AMX was prevalent in P. histicola (5%), P. jejuni (20%), and P. melaninogenica (34%), while 4% of P. jejuni and 3% of P. melaninogenica strains were resistant to AMC. All 144 strains tested were susceptible to ETP, FOX, CLI, and MTZ.

Conclusion: Amoxicillin resistance due to β -lactamase production is common not only among *P. melaninogenica* but also among *P. jejuni* in postpartum women's saliva.

RECOGNITION OF LAMININ BY PATHOGENIC ORAL PREVOTELLA SSP

Marre, A.T.O.;* Boente, R.F.; Ferreira, E.O.; Domingues, R.M.C.P.; Lobo, L.A. Instituto de Microbiologia Paulo de Góes, UFRJ- Universidade Federal do Rio de Janeiro, Brazil

Prevotella is a Gram-negative anaerobic bacteria associated with opportunist infections in oral, vaginal, and gastrointestinal tract. Host tissue adhesion is considered the fundamental step to an infectious process and persistence. P. intermedia, P. melaninogenica, and P. nigrescens require a strong adhesion capacity in the gingival sulcus to initiate colonization and induce an oral disease. Previous studies show that some strains of *P. intermedia* and *P.* nigrescens have a strong affinity for extracellular matrix (ECM) components mediated by the expression of surface proteins, which so far are poorly characterized. This study aims to evaluate the interaction between Prevotella spp. with ECM components and identify the bacterial ligands responsible for this adherence. Laminin was immobilized on glass slides and challenged with different concentrations of bacterial strains. Adherence was quantified by fluorescence microscopy. Initial tests with *P. intermedia* showed no adhesion to laminin. Adherence of *P. nigrescens* increased with inoculums concentration on a dose-dependent manner. An average of 317.4 bacterial per microscope field of view was observed with an inoculums concentration of 108 CFU/ml, whereas, 316.6 for 5x107 108 CFU/ml and 155.4 for 107 CFU/ml. Adhesion to the negative control (BSA) at a concentration of 10⁸ CFU/ml was 98.1. Similar results were observed in *P. melaninogenica*. For identification of bacterial ligands, extraction of outer membrane proteins (OMPs) was performed. Enriched OMP fractions were visualized by SDS-PAGE. Different patterns of OMP was observed among the strains analyzed. These proteins are suitable for screening by chromatography affinity for laminin binding. Our results indicate that the P. intermedia strain tested does binds laminin, in contrast both the *P. nigrescens* and *P. melaninogenica* strains are capable of adhesion to laminin and are suitable for future studies on ligand identification. Our study will allow us to understand the mechanisms involved in bacterial adhesion to host tissues and may help the development of new strategies to prevent this colonization.

Funding: CAPES, FAPERJ and CNPq

IMPROVEMENT OF AN EXPERIMENTAL MODEL OF ORAL BIOFILM

Nguyen, D.;*1 Badet, C.1,2

¹Univ. de Bordeaux, Cours de la Marne, 33082 Bordeaux, France ²Univ. Bordeaux, ISVV, EA 4577 Unité de recherche Oenologie, Institut Polytechnique de Bordeaux, F-33140 Villenave d'Ornon, France

The oral ecosystem exhibits a great complexity, since it provides a habitat for more than 700 different bacterial species. Most of them are organized in a biofilm, both on the dental and on the mucosal surfaces. Studying this complex environment is of utmost importance, because a rupture in its stability can lead to the ascendancy of pathogenic micro-organisms, causing dental decay, gingivitis and periodontitis. However, *in vitro* reproduction of the oral biofilms is extremely difficult because of the complex relations occurring between these numerous species. Besides that, growing, harvesting, and counting the bacteria are three critical laboratory procedures.

Therefore, the aim of our work was to improve an oral biofilm model and to get closer to the *in vivo* conditions. We started improving the static model on hydroxyapatite discs described by Guggenheim (2001). We first modified several culture parameters applying the changes detailed by Ammann *et al.*; we then enhanced the model with our own modifications (for instance, enriched FUM, NAcetylMuramic acid, and hemin were added). Also, in order to get more reproducibility, we improved the bacteria collection method from manually scrapping the discs surface to a precise combination of ultrasonic and mechanical harvest. Moreover, we broadened the techniques used to identify bacteria strains: gram staining, microscopy, molecular techniques with PCR, and mass spectrometry with Maldi-tof technology.

Subsequently, in order to meet our main objective, we developed a dynamic model combining all our previous improvements with a continuous flow of medium and an evacuation of waste. This new protocol allowed us the control of different crucial environmental parameters such as anaerobia, pH & temperature. So, this new model enables the study of environmental variations effects on biofilm development but also a better comprehension of the important factors contributing to a good oral health.

FUNCTIONAL GENOMICS IN AN ORAL CAMPYLOBACTER, CAMPYLOBACTER RECTUS

Threadgill, D.S.;*1.2 Conley, B.A.;² Harrell, E.A.²
¹Texas A&M University, College of Veterinary Medicine and Biomedical Sciences, College Station, TX USA
²North Carolina State University, Raleigh, NC USA

Campylobacter rectus is a rod-shaped Gram-negative anaerobic oral bacterium that has been identified in 90% of adults with initial or established periodontitis, compared with 10% of healthy individuals and 20% of individuals with gingivitis. In addition to a clear association with periodontal disease, recent studies have highlighted the involvement of C. rectus infection in Barrett's esophoagus, oral and extraoral abcesses, inflammatory bowel disease, and adverse pregnancy outcomes in humans including pre-term birth and low birth weight. Despite the frequent isolation of C. rectus from humans, little is known about the molecular mechanisms of pathogenesis associated with this emerging pathogen. To begin functional genomic analysis of virulence genes required for *C. rectus* pathogenesis, we have generated deletion mutations of genes involved in three secretion systems known to be involved in *C. jejuni* pathogenesis. In particular, using Gateway cloning systems or NEB Gibson assembly we have created in ATCC 33238, two mutant strains for a putative type III/flagellar secretion system (T3SS), cellular invasion antigen B-ΔciaB and flagellar filament secretion gene- $\Delta flhB$, one mutant strain for a conserved type IV secretion system (T4SS), Δ*virB9*, and two mutant strains for a conserved type VI secretion system (T6SS), hemolysin coregulated protein- Δhcp and Δ TGH16 and are currently assessing the importance of these secretion systems to the pathogenesis of *C. rectus*. In particular, we are assessing the importance of all the secretion systems to host cell invasion, the importance of the T4SS to DNA uptake and/or transfer, and the T6SS system to pro-inflammatory host cell responses. Previous studies with homologous mutant strains of *C. jejuni*, suggest all of these secretion systems will be very important for the pathogenesis of *C. rectus*. We are creating additional genetic tools for *C.* rectus, including suicide plasmids for complementation and shuttle vectors and are investigating the general use of targeting vectors created for 33238 to additional C. rectus strains.

DETECTION OF FUSOBACTERIUM NECROPHORUM FROM PEDIATRIC PATIENT THROAT CULTURES BY SELECTIVE ANAEROBIC MEDIUM

Van, T.T.;*¹ Cox, L.M.;² Cox, M.E.;² Dien Bard, J.^{1,3}
¹Children's Hospital Los Angeles, Los Angeles, CA USA
²Anaerobe Systems, Morgan Hill, CA USA
³Keck School of Medicine, University of Southern California, Los Angeles, CA USA

F. necrophorum has recently been identified to be an important pathogen of bacterial pharyngitis with clinical presentations indistinguishable from group A *Streptococcus* (GAS) pharyngitis. *F. necrophorum*, however, is not recoverable in routine aerobic throat culture and require specific request for anaerobic culture. This study aimed to determine the prevalence of *F. necrophorum* pharyngitis, using *F. necrophorum* selective media compared to PCR.

Patients presenting to the CHLA emergency department, who had a beta-streptococcus screen test ordered, were enrolled prospectively in the study. Remnant pharyngeal samples were screened for *F. necrophorum* by culture and PCR. Three selective and differential media were included in the primary subculture: egg yolk agar with kanamycin and vancomycin (EYKV), egg yolk agar with kanamycin, vancomycin, and josamycin (EYKVJ), and horse blood agar with josamycin, neomycin, and vancomycin (FSA-H). Colonies were screened for lipase activity on EYKV and EYKVJ and for hemolysis on FSA-H. PCR was performed on all samples and was considered the gold standard.

A total of 196 patients were enrolled, ages 1-20 years (mean: 7.8 y). Compared to PCR, the sensitivity and specificity of the three selective anaerobic media was 100%. *F. necrophorum* was recovered from 8 (4.1%) patients, ages 5-20 years. Of the selective media, *F. necrophorum* was readily detected on EYKVJ with fewer bacterial breakthroughs than EYKV and FSA-H. Although present, hemolysis on FSA-H was not pronounced. Clinically, all 8 patients presented with symptoms commonly associated with GAS pharyngitis, including sore throat (8/8), fever (7/8), and absence of cough (7/8). Only one patient presented with lymphadenopathy and 4 with exudate.

This study shows that *F. necrophrorum* is a clinically relevant cause of pharyngitis, albeit at lower prevalence in pre-adolescent patients. The vast majority of patients positive for *F. necrophrorum* did present with true signs and symptoms of pharyngitis. The selective media were successful in isolating *F. necrophorum* with sensitivity comparable to PCR. EYKVJ had improved inhibition of contaminating oral microflora, thus has the potential as a screening method for *F. necrophorum*.



Wednes	sday, July 13, 2016 Anaerobes in the Genital T	Γract
1300	POSTER SESSION II: INTERACTIONS OF GENITAL TRA ANAEROBES & RELATIONSHIP TO HUMAN DISEASE	
PII-11	Vaginal Microbiotas of Mother-Daughter Pairs Bassis, C.M.;* Alaniz, V.I.; Sack, D.E.; Bullock, K.A.; Lynn, C.S.; Quint, E.H.; Young, V.B.; Bell, J.D.	148
PII-12	Antimicrobial Resistance Rates of <i>Prevotella intermedia</i> and Related Species Isolated from Saliva of Post-Partum Women 10 Years Apart <i>Gürsoy, M.;* Lehto, L.; Könönen, E.</i>	149
PII-13	Pigtail Macaque Vaginal Microbiota Characterized by Culture and Culture-Independent Sequencing Rabe, L.K.;* Ravel, J.; Patton, D.L.; Ma, B.; Gajer, P.; Cosgrove Sweeney, Y.T.; Hillier, S.L.	150
PII-14	Interaction of <i>Gardenerella vaginalis</i> and <i>Vaginotropic lactobacilli</i> in the Distal Reproductive Tract Environmnet <i>Yamamoto</i> , H.S.;* Ryan, S.; Junaid, D.; Luu, N.; Delaney, M.; <i>Onderdonk</i> , A.B.; Fichorova, R.N.	151

VAGINAL MICROBIOTAS OF MOTHER-DAUGHTER PAIRS

Bassis, C.M.;* Alaniz, V.I.; Sack, D.E.; Bullock, K.A.; Lynn, C.S.; Quint, E.H.; Young, V.B.; Bell, J.D.

University of Michigan, Ann Arbor, MI USA

Vaginal bacterial communities play important roles in health. However, the factors that influence their composition and dynamics are not well understood. To better understand the factors that influence vaginal bacterial community structure and dynamics, we compared 13 motherdaughter pairs over 4 weeks. We hypothesize that mothers have a more similar vaginal microbiota to their own daughters than to other women. More specifically, we hypothesize that transmission of vaginal microbiota at birth affects the composition of the vaginal microbiota in adolescence and beyond. If transmission of vaginal microbiota at birth is an important factor for community composition, then we expect that the relationship between mother-daughter vaginal microbiotas will depend on delivery mode. Mother-daughter pairs were recruited from the Pediatric and Adolescent Gynecology clinic at the University of Michigan. Subjects selfcollected vaginal swab samples weekly for 4 weeks and completed an initial questionnaire including gynecologic history and information about their birth. Daughters were 15-20 years old and mothers were 34-57 years old. Three daughters were born by Cesarean section and 10 by vaginal delivery. The V4 region of the bacterial 16S rRNA-encoding gene was amplified from the DNA of 107 vaginal swabs and sequenced with an Illumina MiSeq. The sequences were processed and analyzed using the software package mothur. After clustering the sequences into operational taxonomic units (OTUs) based on sequence similarity, we calculated several ecological metrics, including distances between communities based on relative abundances of both shared and non-shared OTUs. We also investigated the taxonomic composition of the vaginal bacterial communities by classifying sequences using a modified version of the Ribosomal Database Project (RDP). Our preliminary analysis, with an average of 17,184 sequences per sample, suggests that mother-daughter dyads can have highly similar vaginal microbiota, and this can be associated with birth mode. Continued analysis will reveal if the relationship between mother-daughter microbiotas depends on delivery mode or if other factors are important.

ANTIMICROBIAL RESISTANCE RATES OF *PREVOTELLA INTERMEDIA* AND RELATED SPECIES ISOLATED FROM SALIVA OF POST-PARTUM WOMEN 10 YEARS APART

Gürsoy, M.;* Lehto, L.; Könönen, E. Department of Periodontology, Institute of Dentistry, University of Turku, Turku, Finland

Objective: Increasing estradiol concentrations enhance the relative and quantitative enzyme activity of *Prevotella intermedia* group bacteria, including *P. intermedia*, *P. nigrescens*, *P. pallens*, and newly described *P. aurantiaca*, in strain- and dose-dependent manners (Fteita *et al.*, Anaerobe 2015;36:14-8). After delivery, maternal saliva is the most likely vehicle for oral bacteria to be transmitted from mother to child. In this study, *in vitro* resistance to three commonly used antibiotics in dentistry were tested among *P. intermedia* group bacteria collected from saliva of two groups of post-partum women.

Material and Methods: Group I isolates (n=29) were collected from 25 mothers between 1994-1995, and Group II isolates (n=81) from 29 mothers between 2003-2004. Their identification was confirmed by partial 16S rRNA sequencing: *P. nigrescens* (n=52), *P. pallens* (n=41), *P. intermedia* (n=11), and *P. aurantiaca* (n=6). Minimal inhibitory concentrations (MICs) of amoxicillin (AMX), amoxicillin/clavulanate (AMC), and metronidazole (MTZ) were determined using the agar dilution method. Resistance rates were based on the breakpoints provided by EUCAST and CLSI.

Results: In Group I, MIC ranges to AMX were <0.125-16 (*P. intermedia*), <0.125-4 (*P. nigrescens*), and <0.125-16 (*P. pallens*), and to AMC <0.125-4, <0.125-2, and <0.125-8, respectively. In Group II, the ranges to AMX were <0.125-8 (*P. aurantiaca*), <0.125-8 (*P. intermedia*), <0.125-8 (*P. nigrescens*), and <0.125-8 (*P. pallens*), and to AMC <0.125-4, <0.125-1, <0.125-1, and <0.125-2, respectively. According to CLSI breakpoints, the resistance rate of Group I strains to AMX was prevalent in *P. intermedia* (50%), *P. nigrescens* (11%), and *P. pallens* (20%), while the corresponding rate of Group II strains was 60%, 9%, and 19%, respectively. In addition, 2 (33%) of *P. aurantiaca* strains detected in Group II were resistant to AMX, and 8% of *P. pallens* strains to MTZ. The prevalence of intermediate resistance to AMX among *P. pallens* increased from 0% in Group I to 36% in Group II.

Conclusion: Antimicrobial resistance, especially to amoxicillin, is common among salivary *Prevotella intermedia* group bacteria in post-partum women.

PII-13 PII-14

PIGTAIL MACAQUE VAGINAL MICROBIOTA CHARACTERIZED BY CULTURE AND CULTURE-INDEPENDENT SEQUENCING

Rabe, L.K.,*1 Ravel, J.,2 Patton, D.L.,3 Ma, B.,2 Gajer, P.,2 Cosgrove Sweeney, Y.T.,4 Hillier, S.L.1

¹Magee-Womens Research Institute, Pittsburgh, PA USA

²Institute for Genome Sciences, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD USA

³Department of Obstetrics and Gynecology, University of Washington, Seattle, WA USA

⁴Department of Medicine, Division of Infectious Diseases, University of Washington, Seattle, WA USA

Culturomics is a term used to describe evaluation of the microbiota using intense cultivation to detect the broad diversity of microorganisms; DNA from the purified bacteria is extracted and the 16S rRNA gene is amplified, sequenced and used for taxonomic assignment. Our objective was to characterize the microbiota of pig-tailed macaques using culturomics and culture-independent high-throughput sequence analysis of the 16S rRNA gene. Vaginal specimens were collected from 42 sexually mature female Macaca nemestrina (pigtail macaques) housed at the University of Washington. One flocked Eswab was immediately frozen in Amies transport medium and two Dacron swabs were stored in a Port-A-Cul transport tube. DNA was extracted from the flocked swab and the V3-V4 hypervariable region of the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq instrument. The Port-A-Cul swabs were suspended in modified Hanks solution, serially diluted and inoculated onto media for aerobic and anaerobic growth. Each colony type was subcultured and identified by sequencing a 900 basepair portion of the 16S rRNA gene. Sequences were compared to reference strains in GenBank using BLAST and the RDP Taxonomic Classifier. Cultivation yielded 228 unique organisms of which 140 were strict anaerobes and 88 facultative anaerobes or strict aerobes. Of these, 120 (53%) did not match any known species in the BLAST database. Of these 120 putative novel microorganisms, only 75 matched to genus level, 31 to a family, 13 to order, and 1 to phylum only. A comparison between cultivation and culture-independent methods was performed for 36 of the 42 specimens. A total of 82 unique genera or families were identified between the two methods with 20 (24%) detected by culture-independent method only, 37 (45%) detected by cultivation only, and 25 (30%) detected by both methods. An in-depth comparative analysis of sequences is ongoing and may yield greater agreement. Within the genera detected by both methods, there was significant variability in the identification of species. Cultivation and culture-independent methods are complementary for the evaluation of the microbiota of the vagina of pigtail macaques.

INTERACTION OF GARDENERELLA VAGINALIS AND VAGINOTROPIC LACTOBACILLI IN THE DISTAL REPRODUCTIVE TRACT ENVIRONMNET

Yamamoto, H.S.;*1 Ryan, S.;1 Junaid, D.;1 Luu, N.;1 Delaney, M.L.;2 Onderdonk, A.B.;2 Fichorova, R.N.1

¹Laboratory of Genital Tract Biology, Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Brigham and Women's Hospital, Boston, MA USA

²Department of Pathology, Harvard Medical School, Brigham and Women's Hospital, Boston, MA USA

The vaginal bateriome is characterized by relatively high abundance of several known Lactobacillus species, which appear to have different representation among healthy women. In contrast, the high abundance of G. vaginalis shows high specificity for the syndrome of bacterial vaginosis, and its ability to adhere to vaginal epithelial cells provides the scaffold for biofilm formation, allowing other bacteria to become established in this biofilm. We investigated the interaction of highly virulent adherent G. vaginalis with vaginotropic lactobacilli associated with a low inflammatory state in the vaginal environment. Human cervical and vaginal epithelial cells were grown in vitro to forming a confluent epithelial surface and then exposed to G. vaginalis and consecutively or simultaneously to different Lactobacillus species under anaerobic conditions. Bacterial colonization was assessed by epithelium-associated colony forming units (CFU) at different time intervals. Planktonic growth was assessed by CFU and an ATP viability assay. Epithelial innate immune responses were evaluated by NF-κB activation and chemokine production. Only selected lactobacillus isolates e.g. those of *L. crispatus* consistently suppressed the persistence of epitheliacell associated G. vaginalis. L. crispatus and L. gasseri suppressed chemokine responses (e.g. $GRO\alpha$ and $MIP-3\alpha$) to G. vaginalis. The different lactobacillus species showed different capacity to colonize the genital tract epithelial cells, especially in the presence of Gardnerella. A probiotic mix with improved lactobacillus growth in the presence of multiple bacterial species and G. vaginalis was created. Our experimental study suggests that the proportion of different lactobacillus species is critical for shaping the anaerobic vaginal immunobiome.



Wednesday, July 13, 2016

Anaerobic Microbiology

1300	POSTER SESSION II: ANAEROBIC MICROBIOLOGY	
PII-15	Trends in Antimicrobial Resistance among Select Anaerobes from Sterile Sources over a 5-Year Period at an Academic Medical Center	154
	Bourdas, D.;* Hanlon, A.; Tekle, T.; Wakefield, T.; Harris, R.; Simner, P.; Carroll, K.C.	
PII-16	Testing an Antioxidants-Rich Medium to Grow Oral Anaerobes Aerobically	155
	Lange, E.; Melzer-Krick, B.; Henne, K.; Conrads, G.*	
PII-17	The International Anaerobe Quality Assurance Scheme (2) Copsey-Mawer, S.D.;* Morris, T.E.; Hughes, H.	156
PII-18	Survival of Vaginal Microorganisms in Three Commercially Available Transport Systems	157
	DeMarco, A.L.;* Rabe, L.K.; Stoner, K.A.; Austin, M.N.; Avolia, H.A.; Gould, V.A.; Bracken, S.A.; Goldman, J.A.; Hillier, S.L.	
PII-19	Performance of Two Blood Culture Systems to Detect Anaerobic Bacteria: What is the Difference?	158
	Jeverica, S.;* Mueller-Premru, M.; Lampe, T.; Pišek, A.; Nagy, E.	
PII-20	Direct Identification of Anaerobic Bacteria from Positive Blood Culture Bottles Using the Sepsytiper Kit	159
	Mueller-Premru, M.; Jeverica, S.;* Lampe, T.; Pišek, A.; Kostrzewa, M.; Nagy, E.	
PII-21	Host – Pathogens Cross-Talk and the Influence of Host Stress Hormones on Bacterial Virulence Lazar, V.*	160
PII-22	Characterization of the Transcriptional Regulator BmoR in the Oxidative Stress Response of <i>Bacteroides fragilis</i> Teixeira, F.L.;* Pauer, H.; Domingues, R.M.C.P.; Rocha, E.R.;	161
PII-23	Lobo, L.A. Novel Inhibitory Interactions between Prevotella and Streptococcus Species in the Cystic Fibrosis Lung: in silico Informing in vitro Whelan, F.J.;* Waddell, B.; Syed, S.A.; Rabin, H.; Parkins, M.D.; Surette, M.G.	162

Posters will be presented in Poster Session II Wednesday, July 13 1300-1400.

PI-15 PII-16

TRENDS IN ANTIMICROBIAL RESISTANCE AMONG SELECT ANAEROBES FROM STERILE SOURCES OVER A 5-YEAR PERIOD AT AN ACADEMIC MEDICAL CENTER

Bourdas, D.,*1 Hanlon, A.,'1 Tekle, T.;1 Wakefield, T.;1 Harris, R.,1 Simner, P.,2 Carroll, K.C.2

¹Johns Hopkins Hospital, Medical Microbiology Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD USA ²Johns Hopkins Hospital, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD USA

Introduction: Anaerobes cause life-threatening infections among diverse patient populations. Similar to trends in aerobic bacteria, there are reports of increasing resistance to antimicrobial agents among certain species of anaerobes. This study reports susceptibility trends among anaerobes recovered from sterile body fluids and tissues over the last 5 years, when routine testing using the E-test became standard practice at our academic medical center.

Methods: Antimicrobial susceptibility testing was performed and reported following CLSI guidelines. Only one isolate per patient was tested. For the Gram negative anaerobes, the drugs tested and reported include amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotetan, ertapenem, clindamycin, and metronidazole. For the Gram positive pathogens only the results for piperacillin tazobactam, ertapenem, clindamycin, and metronidazole are reported in addition to penicillin.

Results: There were no dramatic changes in resistance profiles among anaerobes from 2010-2014, although susceptibility of the *Fusobacterium* spp. (N=67) declined from 100% to all agents tested to 88-95% with the exception of metronidazole (100% susceptible). The most active agent against all anaerobes was metronidazole (94%-100% susceptible). Activity of the other tested drugs varied by species. For example, cefotetan had poor activity against the *B fragilis* group (33-48% susceptible), especially *B. thetaiotaomicron* (4-8% susceptible), but remained a reasonable choice for *Fusobacterium* spp. (94-100% susceptible). Unlike reports from other centers, very little ertapenem resistance was observed among the *B. fragilis* group (87% in 2010-2011 to 100% in 2013-2014). However, *Clostridium* spp. appeared less susceptible to ertapenem, beginning in 2012 where the susceptibility dropped from 93% prior to 2012 to 74% in 2012 through 2014. This was largely due to an increase in resistance among non-perfringens species, particularly *C. innocuum*.

Conclusions: Susceptibility among anaerobes from sterile sources over a 5 year period at our institution has remained relatively stable, although there is concern regarding the observed decline in ertapenem susceptibility among the *Clostridium* spp.

TESTING AN ANTIOXIDANTS-RICH MEDIUM TO GROW ORAL ANAEROBES AEROBICALLY

Lange, E.; Melzer-Krick, B.; Henne, K.; Conrads, G.* Division of Oral Microbiology and Immunology and Department of Medical Microbiology, RWTH Aachen University Hospital, Aachen, Germany

The aerobic growth of strictly anaerobic or microaerophilic and fastidious bacterial species, including many of the genera Clostridium and Bacteroides, together with Parvimonas micra, Prevotella buccalis, Veillonella parvula, Campylobacter spp., and Haemophilus spp, in a universal culture medium containing a combination of antioxidants, was very recently demonstrated (Dione-N et al. Clin. Microbiol Infect. 2016 Jan;22(1):53-8). This medium is based on Schaedler agar, supplemented with 1 g/L ascorbic acid, 0.1 g/L glutathione, 0.4 g/L uric acid, 0.1 g/L hemin, and 2 g/L α -ketoglutarate. We tested aerobically the culture of 50 bacterial strains from 25 bacterial species, known to prefer capnophilic or anaerobic conditions. Among them are marker bacteria for periodontitis, especially Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and Tannerella forsythia, as they were not included in the panel of strains tested before. We also subjected samples of freshly collected gingival sulcus fluid and saliva. We coupled the growth with MALDI-TOF analysis and/or 16S rRNA-gene amplification and sequencing for identification.

Our study started at the time of call for ASA abstracts. First results, however, look promising. Experiences and especially challenges from the perspective of a laboratory specialized on oral anaerobes will be presented and discussed in depth.

THE INTERNATIONAL ANAEROBE QUALITY ASSURANCE SCHEME (2)

Copsey-Mawer, S.D.;* Morris, T.E.; Hughes, H. UK Anaerobe Reference Laboratory (UKARU), Public Health Wales, Cardiff, UK

Introduction: Diagnostic laboratories can participate in existing external quality assurance schemes to cover most tests, but there is still no such scheme for anaerobe characterisation at the reference laboratory level. With the advent of more stringent standards such as ISO 15189, a gap still exists in terms of assuring the quality and robustness of the characterisation of anaerobic bacteria.

Objectives: To re-launch a modern international anaerobe quality assurance scheme (IAQAS) for monitoring the quality of anaerobic identification, susceptibility testing, and molecular characterisation. Initially this will be for colleagues from similar specialist/reference laboratories. Expansion to other laboratories will be considered once the scheme is reestablished.

Methods: Laboratories specialising in the characterisation of anaerobic bacteria are asked to participate in the scheme with view that isolates will be shared between these centres on an annual basis. Interested parties are requested to sign up online via the UKARU website. Distribution of the first round of strains is estimated for late 2016.

A multifactorial scoring system will be used taking into account the accuracy of identification, susceptibility testing, and further molecular characterisation (such as resistance markers and insertion elements). The use of up to date taxonomy will also be considered as part of the scheme.

Funding for this scheme will be sought via the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Anaerobic Infections (ESGAI).

Results and Discussion: All results will be submitted via the ARU website and regular teleconference/videoconference meetings will be setup to review and discuss these results. The re-launch of the IAQAS will enable laboratories to evaluate their quality assurance using a suitable EQA scheme. It will also serve to establish valuable lines of communication between laboratories and enhance the sharing of knowledge within the anaerobe community.

SURVIVAL OF VAGINAL MICROORGANISMS IN THREE COMMERCIALLY AVAILABLE TRANSPORT SYSTEMS

DeMarco, A.L.;*1 Rabe, L.K.;1 Stoner, K.A.;1 Austin, M.N.;1 Avolia, H.A.;1 Gould, V.A.;1 Bracken, S.A.;1 Goldman, J.A.;1 Hillier, S.L.,12 1Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA USA

²University of Pittsburgh Department of Obstetrics, Gynecology and Reproductive Sciences, Pittsburgh, PA USA

Transport systems are used to collect and maintain the viability of microorganisms. Starswab[®] Anaerobic Transport System (Glass), BD CultureSwab[™] MaxV (+) Amies Medium without Charcoal (MaxV), and Fisherfinest® with Amies gel Transport Medium without charcoal (Amies) were evaluated for their capacity to maintain the viability of anaerobic and aerobic bacteria commonly found in the vagina. Two sets containing mixtures of microorganisms representing women with Lactobacillus predominant microbiota [Lactobacillus crispatus (LC), Lactobacillus jensenii (LJ), group B streptococci, Escherichia coli (EC), Enterococcus spp., and Candida albicans (CA)] and women with bacterial vaginosis [Lactobacillus iners, Atopobium vaginae (AV), Gardnerella vaginalis (GV), Prevotella timonensis (PT), Prevotella bivia (PB), Porphyromonas uenonis, Peptoniphilus harei, Mobiluncus curtisii (MC), and Mycoplasma hominis (MH)] were prepared. It has previously been established that some organisms propagate at 24°C, therefore single swabs were inoculated in triplicate for each set and held at 4°C for 24, 48, 72, and 96 hours (hrs). Following each time point, swabs were serial diluted, inoculated onto selected media, incubated, and each colony type was quantified and identified. Loss of viability was reported if ≥ 1 log decrease in growth was observed and the concentration either remained constant or continued to decrease through the remaining time points. At 24hrs, a loss of viability occurred for GV in both Amies and Glass transporters and for PB, MC, and LJ in MaxV. At 48hrs, a loss of viability occurred for PB, LJ, EC, and CA in Amies, AV and EC in Glass and GV in MaxV. Stability of all other microorganisms occurred for all transporters through 72hrs. At 96hrs, a loss of viability occurred for AV, PT and LC in Amies and AV in MaxV. It should be noted that $a > 2 \log$ decrease was never observed in glass but occurred for LJ at 72hrs in MaxV and AV, GV, and PB in Amies and AV, MC, and MH in MaxV at 96hrs. With the exception of AV (-1.5 log at 96hrs), viability, and stability of vaginal microorganisms was maintained in the Glass transporter compared to MaxV and Amies.

157

PERFORMANCE OF TWO BLOOD CULTURE SYSTEMS TO DETECT ANAEROBIC BACTERIA: WHAT IS THE DIFFERENCE?

Jeverica, S.;*1 Mueller-Premru, M.;1 Lampe, T.;1 Pišek, A.;1 Nagy, E.2 ¹Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

²Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary

We studied the performance of two blood culture (BC) bottles/systems, (i) BacT/ALERT-FN Plus (BioMerieux) and (ii) BACTEC-Lytic (Becton Dickinson) for the detection and the time-to-positivity (TTP) against a balanced panel of anaerobic bacteria (48), comprising 19 reference strains and 29 most common clinical species isolated from BCs in a major Slovenian tertiary-care hospital.

The bacterial panel included *Bacteroides* spp. (10), *Prevotellae* spp. (8), GPAC (8), Clostridium spp. (8), Fusobacterium spp. (5), Eggerthella spp. (2), Actinomyces spp. (2), Propionibacterium spp. (2), Porphyromonas spp. (1), Veillonella spp. (1), and Lactobacillus spp. (1). Standard suspension of bacteria were inoculated to BC bottles (cca. 1000 CFU/bottle) in duplicates. Five mL of defibrinated sterile horse blood was added to simulate blood culture condition. Concentration of inoculated bacteria was controlled. Overall, 70.8% (34) and 79.2% (38) of strains were detected in BacT/ALERT-FN Plus and BACTEC-Lytic BC bottles, respectively (p=0.38). Among Gram negative and Gram positive anaerobes, the detection rate was 76.0% vs. 92.0% (p=0.22) and 65.2% vs. 65.2% (p=1), respectively. The average TTD was calculated for strains detected by both BC bottles (30) and was 40.85 h and 28.08 h for BacT/ALERT-FN Plus and BACTEC-Lytic BC bottles, respectively (p<0.001). The mean difference was 12.76 h (95% CI: 6.21-19-31 h). Among strains not detected by any BC bottles/systems were Porphyromonas gingivalis, Finegoldia magna, Peptostreptococcus anaerobius, Propionibacterium acnes, and two clostridia, C. novy and C. clostridiforme. Eggerthella lenta and Prevotella bivia were detected only by BacT/ALERT-FN Plus, while Prevotella disiens, Prevotella intermedia and Prevotella melaninogenica were detected only by BACTEC-Lytic BC bottles/systems.

Anaerobic bacteria represent a minority of positive BC isolates. However, far from ideal detection rate was observed in this study for both tested BC bottles/systems. Nevertheless, BACTEC-Lytic was superior to BacT/ALERT FN Plus with 12 h shorter TTD. The impact of lack of antimicrobial scavenger resins in BACTEC-Lytic BC bottles on clinical detection rate of anaerobic bacteria remains to be determined.

DIRECT IDENTIFICATION OF ANAEROBIC BACTERIA FROM POSITIVE BLOOD CULTURE BOTTLES USING THE SEPSYTIPER KIT

Mueller-Premru, M.;¹ Jeverica, S.;*¹ Lampe, T.;¹ Pišek, A.;¹ Kostrzewa, M.;² Nagy, E.³

¹Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia;

²Bruker Daltonik, Bremen, Germany

³Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary

We evaluated performance of the commercial IVD Sepsityper kit (Bruker Daltonics) for rapid identification of anaerobic bacteria directly from positive blood culture (BC) bottles of two types: (i) BacT/ALERT-FN Plus (bioMerieux) and (ii) BACTEC-Lytic (Becton Dickinson).

In total, 48 reference and clinical strains of anaerobic bacteria (i.e. Bacteroides spp. [10], Prevotellae spp. [8], GPAC [8], Clostridium spp. [8], Fusobacterium spp. [5], Eggerthella spp. [2], Actinomyces spp. [2], Propionibacterium spp. [2], Porphyromonas spp. [1], Veillonella spp. [1], and Lactobacillus spp. [1]) were inoculated in duplicates to both tested BC bottles/systems. Following signaled positivity or after 5-days incubation, the Sepsityper kit was used for specimen preparation and MALDI-TOF (Bruker Daltonics) analysis. Overall, 34 (70.8%) and 38 (79.2%) were detected with BacT/ALERT-FN Plus and BACTEC-Lytic BC bottles/system, respectively. Of those, correct identification (score >1.6) was achieved for 25 (73.5%) strains from BacT/ALERT-FN Plus and 22 (57.9%) strains from BACTEC-Lytic bottles (p=0.16). Among Gram negative/positive anaerobes, correct identification was achieved for 13 (68.4%)/12 (80%) strains from BacT/ALERT-FN Plus and 11 (47.8%)/11 (73.3%) from BACTEC-Lytic bottles, respectively. Among 10 Bacteroides spp. strains tested, 4 (40%) were not identified from BacT/ALERT-FN Plus bottles and 8 (80%) from BACTEC-Lytic bottles.

In the present study, suboptimal detection rate of anaerobic bacteria was achieved for the two tested BC bottle/systems. Direct identification from positive BC bottle using the Sepsityper kit and MALDI-TOF gave at best 73.5% correct identification and did not significantly differ between the two tested BC bottles. Direct identification seems to be less reliable for anaerobes vs. aerobes and for Gram negative vs. Gram positive anaerobes. Anaerobes represent a minority of isolates from blood; however, a majority of *Bacteroides* spp., the most common anaerobic isolate from blood, were not identified reliably in this study.

HOST – PATHOGENS CROSS-TALK AND THE INFLUENCE OF HOST STRESS HORMONES ON BACTERIAL VIRULENCE

Lazar, V.*

University of Bucharest, Faculty of Biology, Microbiology, Immunology Department, Bucharest, Romania

For a long time, bacteria were considered to live as single cells, but almost three decades ago microbiologists definitively established that bacteria are living in large communities or biofilms. The biofilm embedded cells communicate by fifferent small diffusible molecules or autoinducers (AIs) that act as signals, such as oligopeptides in Gram positive bacteria, acylhomoserine-lactones in Gram negative ones, and also some universal molecules, recognized by both bacteria – AIs-2. These molecules are mediating the quorum sensing (QS) mechanism, a cell density-dependent system that is used to coordinate gene expression and adaptation to local conditions and to regulate genes that promote bacterial adherence and biofilm formation, host colonization, and other virulence genes in pathogenic bacteria. More recently, it is also proved that bacteria are able to recognize and respond to signal molecules produced by host too, such as hormones, at present being a great interest on deciphering host-pathogens cross-talk during infection, in order to interfere with it and discover new antipathogenic strategies.

The purpose of this study: investigation of the influence of the stress hormone noradrenaline (NA) on the physiology and behavior of the opportunistic pathogen *Pseudomonas aeruginosa*, using phenotypic and molecular methods. The viable cell count results proved that NA is able to promote growth only in serum-containing minimal medium, displaying a toxic effect in serum-free minimal medium, because of its iron binding affinities, as revealed by ICP-MS analysis.

The iron-uptake mechanism was also involved in the NA effects on QS controlled phenotypes as swarming motility, pyoverdine and pyocyanine production, and also biofilm formation.

NA has proved to stimulate *P. aeruginosa* growth by an endogenous siderophore-independent mechanism, promoting the growth of a siderophore deletion mutant (*P. aeruginosa PAOI*), by acting itself as an exogenous pseudosiderophore. Nevertheless, our molecular data suggest that NA interferes with bacterial ferrienterobactin iron uptake. These results demonstrate that iron uptake modulation represents a key element in NA signaling in *P. aeruginosa*.

CHARACTERIZATION OF THE TRANSCRIPTIONAL REGULATOR BMOR IN THE OXIDATIVE STRESS RESPONSE OF BACTEROIDES FRAGILIS

Teixeira, F.L.;*1,2 Pauer, H.;1,2 Domingues, R.M.C.P.;1 Rocha, E.R.;2 Lobo, L.A.1 IMPG/UFRJ, Rio de Janeiro, Brazil 2East Carolina University, Greenville, NC USA

Bacteroides fragilis is one the most aerotolerant species among strict anaerobes, surviving for up to 72h when exposed to atmospheric oxygen. The oxidative stress response (OSR) affects nearly half of its whole genomic expression. Except for the peroxide response regulator OxyR, the regulation of this OSR remains largely uncharacterized. The aim of this study is to evaluate the role of BmoR, a new oxidative stress related regulator and to compare its activity to OxyR, a well-established regulator. Mutant strains were constructed and used in phenotypic and molecular assays. Oxidative stress assays showed that BmoR has an impact on tolerance to low levels of oxygen, but its role on survival during extended period of exposure to oxygen is not completely understood. No significant difference in survival was seen in the $\Delta bmoR$ following oxygen exposure compared to wildtype strain. However, there was an increase in the survival rate in the $\Delta bmoR\Delta oxyR$ double mutant compared to the $\Delta oxyR$ mutant. Microarray analysis of whole genome expression in oxygen stressed cells showed that in the absence of *bmoR* there was significant up-regulation of thioredoxin C (trxC) and a putative pyridine nucleotide oxidoreductase (PNOR), the latter located immediately upstream of bmoR. Quantitative PCR was used to confirm the microarray results. Interestingly, expression of trxC in $\Delta bmoR\Delta oxyR$ is in an intermediate level between the expressions of the respective single mutants, which suggests a possible overlap of both BmoR and OxyR regulons and could explain the results of the phenotypic assays. Electrophoretic mobility shift assays showed that BmoR binds to a promoter region upstream of the oxidoreductase gene suggesting that PNOR and bmoR are expressed as a bicistronic mRNA. Further studies are been carried out to understand the mechanisms involved in the control of this complex OSR in *B. fragilis*. These results show that the OSR is a potential target for developing new strategies for intervention and control of infectious disease, in view of the alarming increase in resistance to antibiotics seen in this species.

NOVEL INHIBITORY INTERACTIONS BETWEEN PREVOTELLA AND STREPTOCOCCUS SPECIES IN THE CYSTIC FIBROSIS LUNG: IN SILICO INFORMING IN VITRO

Whelan, F.J.;*¹ Waddell, B.;² Syed, S.A.;¹ Rabin, H.;² Parkins, M.D.;² Surette, M.G.¹

¹McMaster University, Hamilton, ON Canada

²The University of Calgary, Calgary, AB Canada

A plethora of diverse microbes adapt to fit ecological niches within highly variable environments. As in any ecosystem, when multiple organisms rely on common resources, competition ensues; thus, many bacterial species have developed mechanisms in order to compete with each other for limited space and resources. 16S rRNA gene sequencing profiles the presence and relative abundance of bacteria in a given environment, such as those associated with the human body. In this study, we conducted 16S rRNA sequencing and extensive bacterial culture of sputum samples obtained from Cystic Fibrosis patients, in order to study the complex polymicrobial communities within the lung environment.

The purpose of this study was to predict patterns of bacterial cooccurrence and co-exclusion using in silico techniques, in order to inform further *in vitro* competition assays and investigations. By incorporating a bioinformatic approach, we were able to narrow our focus to 12 inhibitory and 3 co-occurring bacterial pairs predicted based on in silico analyses of cultured communities. Using these predictions, competition was measured in vitro using spot assays and bacterial overlays on solid agar, as well as mixed broth cultures. Testing these interactions lead to the identification of bacterial competition between Streptococcus intermedius and Prevotella melaninogenica species. This competition is dependent on the culturing conditions used, suggesting that this interaction is resource-dependent. Further, this interspecific competition is dependent on a particular *P*. melaninogenica strain but occurs with all S. intermedius strains that have been tested to date. Ongoing studies using a S. intermedius transposon library will aid in determining the mechanism of inhibition between these two species. Understanding these interactions will be valuable in further characterizing the importance of anaerobic species in Cystic Fibrosis lung infections.

1300	POSTER SESSION II: PROBIOTICS	
PII-24	Inhibitory Effect of the Probiotic <i>Lactobacillus delbrueckii</i> subsp. <i>Bulgaricus</i> G-LB-44 on Pathogenic Organisms Grown in Organic Juice and Produce	164
	DuBois, A.M.;* Delaney, M.L.; DuBois, G.M.; Onderdonk, A.B.	
PII-25	Lactobacillus reuteri Decreases Adherent Tumor Mucins and Enhances Chemotheraputic Susceptibility	165
	Engevik, M.A.;* Luk, B.K.; Ganesh, B.P.; Hall, A.; Versalovic, J.	
PII-26	Probiotics and C. Difficile Infection (CDI)	166
	Goldstein, E.J.C.;* Johnson, S.J.; Louie, T.J.; Maziade, P-J.; Millette, M.; Sniffen, J.C.	
PII-27	Theoretical Aspects for Practical Applications of Intestinal Lactobacillus Species Strains	167
	Mikelsaar, M.;* Štšepetova, J.; Mändar, R.; Songisepp, E.; Sepp, E.	
PII-28	Bio-K+ Probiotic Strains Reduce Toxin and Spore-Forming Gene Expression in <i>Clostridium difficile</i>	168
	Paquette, P.; Frappier, M.; Auclair, J.; Gunaratnam, S.; Millette, M.*	
PII-29	In vitro Investigations of Innovative Fructooligosaccharides: Modulations of Probiotics, Enteropathogens and Colic Cell Proliferation	169
	Grimoud, J.; Ouarné, F.; Gignac-Brassard, S.; Roques, C.*	
PII-30	Isolation of Lactic Acid Bacteria from Oysters (<i>Crassostrea gigas</i>) for Their Potential Use as Probiotics Kang, C.H.; Shin, Y.J.; Kim, W.R.; Jang, S.C.; Gu, T.; Jung, Y.; So, J.S.*	170
PII-31	New Functional Foods with Probiotics and Prebiotics: Perspectives in the Control of Obesity	171
	Torriani, S.*	

INHIBITORY EFFECT OF THE PROBIOTIC *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* G-LB-44 ON PATHOGENIC ORGANISMS GROWN IN ORGANIC JUICE AND PRODUCE

DuBois, A.M.;*¹ Delaney, M.L.;¹ DuBois, G.M.;² Onderdonk, A.B.¹ Brigham and Women's Hospital, Department of Pathology, Boston, MA USA ²Western New England University, Springfield, MA USA

The inhibitory effect of probiotic strain, *Lactobacillus delbrueckii* subsp. *bulgaricus* G-LB-44 (LB) was evaluated using organic juice and produce containing potentially pathogenic organisms.

 1×10^7 CFU/ml LB was added to organic juice containing 1×10^2 to 1×10^5 CFU/ml of bacteria most commonly associated with food borne illness. Test samples and control samples (no LB) were refrigerated overnight followed by incubation for 48 hours and the bacterial concentrations were determined. Test samples containing various strains of *Acinetobacter baumanii*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella sonnei*, and *Staphylococcus aureus* were completely inhibited with an overall 6 to 8 log reduction in concentration when compared to control samples. The following were markedly inhibited: *E.coli* O157:H7 (3.3 \times 10¹ vs 2.1 \times 108 CFU/ml), *Enterococcus faecalis* (3.6 \times 106 vs 5.1 \times 108) and *E. faecium* (3.5 \times 106 vs 1.1 \times 109).

The total bacterial load present in organic spinach and kale purchased from a natural food supermarket was $1.4 \times 10^8 \, \text{CFU/g}$ spinach and $2.9 \times 10^5 \, \text{CFU/g}$ kale. The produce contained numerous Gram-positive and negative environmental species. Portions of the spinach and kale were emulsified and incubated or refrigerated with $1 \times 10^7 \, \text{or} \ 1 \times 10^8 \, \text{CFU/ml} \, \text{LB}$ or no LB. At 24, 48, and 72 hours bacterial concentrations were determined. After 72 hours of incubation with $1 \times 10^8 \, \text{CFU/ml} \, \text{LB}$, both spinach and kale had a 6 log reduction in the total bacterial concentration versus no LB. After 72 hours of refrigeration with $1 \times 10^8 \, \text{CFU/ml} \, \text{LB}$, spinach and kale had a 3 and 2 log reduction, respectively versus no LB. Incubation or refrigeration with $1 \times 10^8 \, \text{CFU/ml} \, \text{LB}$ showed a minimal reduction in the total amount of bacteria.

These results show that LB can completely inhibit or reduce the growth of potentially harmful bacteria that cause disease, as well as reduce the bacterial load present on organic produce. The broad based activity of LB suggests the presence of unique cellular components.

LACTOBACILLUS REUTERI DECREASES ADHERENT TUMOR MUCINS AND ENHANCES CHEMOTHERAPUTIC SUSCEPTIBILITY

Engevik, M.A.;*1.2 Luk, B.K.;1.2 Ganesh, B.P.;1.2 Hall, A.;1.2 Versalovic, J.1.2 Department of Pathology & Immunology, Baylor College of Medicine, Houston, TX USA

²Department of Pathology, Texas Children's Hospital, Houston, TX USA

Background: Colorectal cancer (CRC) is the second leading cause of cancer related deaths in the United States. Although CRC is commonly treated with the chemotherapeutic agent Fluorouracil (5-FU), only 15% of advanced CRC tumors respond. Mucins, a hallmark of epithelia derived carcinomas, have been hypothesized to limit the uptake of the chemotherapeutic agents. Select gut microbes are capable of altering mucins and may increase chemotherapy uptake in resistant cancers.

Methods & Results: HT29, T84, and HT-29-MTX-E12 cell lines were incubated with the probiotic human gut microbe Lactobacillus reuteri conditioned media (CM). L. reuteri CM decreased MUC1, MUC4 and MUC5AC gene expression and protein. To assess whether bacterialspecific changes in mucin expression correlated with increased cancer cell susceptibility to drug treatment, HT29, T84, and HT29-MTX-E12 cells were incubated with CM for 3 hours followed by the chemotherapeutic agent 5-FU. L. reuteri CM increased metabolic activity as determined by resazurin and cell cycle analysis. Magpix multiplex assay revealed that L. reuteri CM promoted ERK, CREB, and JNK phosphorylation. No changes were observed in p7056, STAT3, STAT5, p38 or NF- B. Furthermore, L. reuteri CM enhanced susceptibility to 5-FU treatment resulting in decreased cancer cell viability as determined by resazurin, trypan blue, and Annexin V staining. This effect was independent of lactate. The *L. reuteri* secreted factor was found to be a >10 kDa, heat stable protein. To establish the ability of *L. reuteri* to promote tumor 5-FU susceptibility in vivo, C57BL/6J-Apc^{Min}/J mouse tumors were excised and ex-vivo treated with L. reuteri CM followed by 5-FU. Addition of CM resulted in enhanced susceptibility of male and female intestinal tumors to 5-FU treatment. ApcMin mice colonized with L. reuteri bacteria had significantly decreased adherent Muc1, Muc4, and Muc5ac tumor masses, consistent with cell line data.

Conclusion: Together this data suggests that bacterial-induced modifications of cancer-driven mucus in combination with traditional chemotherapeutic agents may provide new therapeutic strategies for the treatment of chemoresistant cancer.

PII-26 PII-27

PROBIOTICS AND C. DIFFICILE INFECTION (CDI)

Goldstein, E.J.C.;*1 Johnson, S.;2 Louie, T.J.;3 Maziade, P-J.;4 Millette, M.;5 Sniffen, J.C.6

¹R.M. Alden Research Laboratory, Santa Monica, CA USA

²Loyola School of Medicine, Chicago IL USA

³University of Calgary, Calgary, AB Canada

⁴Pierre-Le Gardeur Hospital, Terrebonne, QC Canada

⁵Bio-K Plus International Inc., Laval, QC Canada

⁶Florida Hospital, Orlando FL USA

Probiotics are increasingly given to patients on antibiotics in US hospitals; those with CDI are 21 times more likely to receive probiotics. The hypothesis that probiotics can help prevent CDI is supported by the observation that lactobacilli remain present during and after the treatment of CDI. There is a plethora of agents available and only limited control trial data available. Bio-K+, a combination of *L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 has shown both efficacy and safety in controlled trials and retrospective reviews. We review some supporting data of this probiotic for CDI prevention.

These strains show antimicrobial activity against *C. difficile* and toxin neutralization capacity *in vitro*. An 11 study meta-analysis for CDI prevention (Johnson *et al*, 2012) noted that CDI could be prevented by specific probiotic strains. Gao *et al*. (2010) in a randomized, controlled, double blind placebo controlled trial studied Bio-K+ in a high risk population in a Shanghai hospital. Prophylaxis began within 36 h of initial antibiotic administration, continued for 5 days after the last antibiotic dose, and pts were followed for an additional 21 days. The probiotic group had a lower Antibiotic Associated Diarrhea incidence vs. placebo (15.5% vs 44.1%) and a lower CDI incidence vs. placebo (1.2% vs 23.8 %). Other trials have shown efficacy trends but not statistical significance.

In an observational study, at a 284 bed Canadian community hospital, Bio-K+ has been given to > 44,000 patients who received antibiotics. During that time the CDI rate declined from 18.0 to 2.3 cases per 10,000 pt days, which was lower than similar regional hospitals. In the retrospective review, no cases of *Lactobacillus* bacteremia were observed.

A panel of experts proposed a "pathway to prevent nosocomial CDI" and concluded that "probiotics should be added in the bundle of preventative measures to control" CDI. This is in accord with Health Canada's approval of this product for risk reduction of CDI in hospitalized patients.

166

THEORETICAL ASPECTS FOR PRACTICAL APPLICATIONS OF INTESTINAL *LACTOBACILLUS* SPECIES STRAINS

Mikelsaar, M.;*¹ Štšepetova, J.;¹ Mändar, R.;¹ Songisepp, E.;² Sepp, E.¹ Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

²Bio-Competence Centre of Healthy Dairy Products, Tartu, Estonia

Theoretical aspects for practical applications of *Lactobacillus* sp. with its large metabolic functions need extensive comparison between the obtained phylogenetic and phenotypic data in elaboration of probiotics. The diversity of intestinal lactic acid bacteria of *Firmicutes* phyla can be proved with taxonomical, functional, and host-microbial interactions. The promotive role of metabolites of the three fermentative groups of Lactobacillus sp. on microbial ecosystem is performed by intertwined increase of beneficial compounds like acetate, propionate, butyrate, NO produced by other groups of intestinal bacteria. On the other hand, the suppressive impact of Lactobacillus sp. for reduction of detrimental bacterial metabolites, such as ammonia, indole, para-cresol, sulfides, H₂, reactive oxygen or nitrogen species and their producers has also been demonstrated. Both double (promotive, suppressive) functions of lactobacilli are reviewed according in vitro, experimental animal studies and human trials. The applied beneficial strains of lactobacilli increase the abundance and diversity of indigenous Lactobacillus sp. By similar increase of some other members of microbiota, this supports the correction of the impaired balance of microbiota.

The lactobacilli are closely bound to host genetic biomarkers and are driven by epigenetic influences, such as age, diet, social-economic factors, persistent infections, and metabolic disorders. Individual specificity of colonization by *Lactobacillus* strains with particular metabolic activity forms the basis for biodiversity of human *Lactobacillus* sp. and affords the selection of specific strains with evidence based functional properties. The research experience obtained largely by both phylogenetic and phenotypic studies of *Lactobacillus* communities, the newly discovered functional properties of specific strains and correlations with host biomarkers have offered possibilities for drawing several theoretical conclusions and practical applications for improvement of gut microbiota composition with *Lactobacillus* sp. probiotics in maintenance of human health.

BIO-K+ PROBIOTIC STRAINS REDUCE TOXIN AND SPORE-FORMING GENE EXPRESSION IN CLOSTRIDIUM DIFFICILE

Paquette, P.; Frappier, M.; Auclair, J.; Gunaratnam, S.; Millette, M.* Bio-K Plus International Inc., Laval, QC Canada

Clostridium difficile (CD) has been associated with outbreaks of diarrhea in hospitals and long-term care facilities and has been recognized as the main cause of antibiotic-associated diarrhea (AAD) in hospitalized patients. CD infection (CDI) has placed a huge financial burden on the US healthcare system since past decade. Besides, The Centers for Disease Control and Prevention recently identified CDI as an "urgent threat", highlighting the need for immediate and aggressive action to prevent this infection.

Clinical trials have demonstrated the capacity of a specific probiotic product named Bio-K+® containing *Lactobacillus acidophilus* CL1285®, *L. casei* LCB80R® and *L. rhamnosus* CLR2® to reduce the incidence of AAD and CDI in hospitalized adults receiving antibiotics. Lactobacilli can achieve this preventive effect by many well-known and non-specific mechanisms such as organic acids, bacteriocins and hydrogen peroxide production, which can directly impair CD growth. Despite this, we hypothesized that Bio-K+ probiotic strains can specifically inhibit CD virulence factors such as toxin production and spore-forming pathways.

In this study, the effect of Bio-K+ probiotic strains on the expression of some virulence factors when co-cultured in TY broth (previously demonstrated to prevent medium acidification) in the presence of CD strain R20291 was investigated. To achieve this objective, RT-qPCR strategy to quantify transcript-level gene expression associated with toxin production (*tcdA* and *tcdB*) and spore-forming pathways (*spoIIR* and *spoIIID*) was developed.

Results demonstrated that in pure culture, the lactobacilli did not thrive nor acidify on TY broth while CD strain R20291 grew well and showed normal gene expression. However, when CD was placed in the presence of each lactobacilli or a mixture, a decline of growth rate and downregulation of *tcdA*, *tcdB*, *spoIIR*, and *spoIIID* gene expression, as well as few housekeeping genes was observed. Altogether, these results suggest that Bio-K+® probiotic strains may interfere with CD pathogenesis, adding new knowledge about CDI prevention observed in clinical trials.

IN VITRO INVESTIGATIONS OF INNOVATIVE FRUCTOOLI-GOSACCHARIDES: MODULATIONS OF PROBIOTICS, ENTEROPATHOGENS AND COLIC CELL PROLIFERATION

Grimoud, J.;¹ Ouarné, F.;² Gignac-Brassard, S.¹; Roques, C.*¹ ¹Université Paul Sabatier, Laboratoire de Génie Chimique, Faculté de Pharmacie, Toulouse, France ²CRITT Bio Industries, INSA, Toulouse, France

Most of fructooligosaccharides (FOS) are produced by enzymatic synthesis from sucrose or by inulin hydrolysis. We investigated *in vitro* the prebiotic characteristics of original FOS which differ from already commercialized ones by a transitional polymers residues composition and length.

Anaerobic lactic acid bacteria, belonging to the genus *Bifidobacteria*, Lactobacillus, Lactococcus, and Pediococcus were used to evaluate the ability of original FOS to promote growth of gut beneficial strains. Raftilose[®]L95 and Actilight®950P were used as commercialized reference FOS (from inulin hydrolysis and chemical synthesis respectively). Bacteria growths were monitored in medium with the tested carbohydrates as sole carbon source. Growth parameters showed that innovative FOS were metabolized by all probiotics, at the same rates that those observed with Actilight®950P, and higher level than Raftilose®L95 since only 3 strains used this last FOS. HPLC analyses were conducted on supernatant to check the consumption of each degree of polymerisation (DP). Short DP (DP2 and DP3) were preferentially consumed, while bifidobacteria were also able to metabolize DP4. Several enteropathogen growths were also checked when co-cultured with probiotics and carbon sources used in the previous assay. One pathogen and one lactic acid bacteria were inoculated at equal concentration in a basal medium with the selected carbohydrates. At the end of the incubation time, the both strains were enumerated on selective agar. Among the tested pathogens, Candida albicans and Clostridium difficile were efficiently inhibited by some lactobacilli and bifidobacteria with innovative FOS as sole carbon source. These inhibitions were significantly more important than those obtained with Raftilose[®]L95. Finally we monitored the anti-proliferative effect of our compounds on human colic epithelium cancer cells HT-29 by an XTT assay. None of the prebiotic or probiotics induced a significant effect. On the other hand, the tested strain of Bifidobacterium breve, only when cultured on innovative FOS, lead to a dramatic decrease in cancer cells proliferation, reaching an inhibition of 80 %.

We demonstrated here *in vitro* that innovative evaluated FOS are very well metabolized by beneficial gut bacteria at equal or higher rates than commercialized FOS. They also could inhibit more efficiently *C. albicans* and *C. difficile*, some major pathogens involved in antibiotic-associated diarrhoea. In addition, only these innovative FOS were able to reduce significantly HT-29 cancer cells proliferation in combination with the probiotic *B. breve*. Therefore, innovative FOS are good candidates to promote beneficial gut bacteria and could be further evaluated in *in vivo* models for beneficial effects in gastrointestinal pathologies, especially for colorectal cancers.

ISOLATION OF LACTIC ACID BACTERIA FROM OYSTERS (CRASSOSTREA GIGAS) FOR THEIR POTENTIAL USE AS PROBIOTICS

Kang, C.H.; Shin, Y.J.; Kim, W.R.; Jang, S.C.; Gu, T.; Jung, Y.; So, J.S.*
*Department of Biological Engineering, Inha University, Incheon, Korea

In this study, we isolated lactic acid bacteria (LAB) from the oyster (Crassostrea gigas) and selected several environmental stress-resistant isolates for the development of a future probiotic adjuvant for marine aquaculture. Twenty-six presumptive LAB strains were isolated from oysters and screened by an agar diffusion assay for antimicrobial activity against three marine pathogens: Vibrio parahaemolyticus, Streptococcus iniae, and Edwardsiella tarda. Eight isolates were found to have antibacterial activity against V. parahaemolyticus; in particular, 5 isolates showed a growthinhibitory activity with inhibition zone diameters >15 mm. Five isolates (JL17, JL18, JL28, HL7, and HL32) were also antagonistic against S. iniae and E. tarda. Out of the 5 isolates HL7 was selected as a potential probiotics because of its high resistance to environmental stressors: it showed the minimal inhibitory concentration of NaCl 1.9 M, ethanol 11%, and hydrogen peroxide 0.013%. HL7 was molecularly identified as Entercoccus faecium by 16S rRNA gene sequencing (Genbank accession numbers KR153314). E. faecium HL7 was bile resistant (maintained 50% viability at 0.45% bile salt w/v) and acid resistant (maintained 40% viability at pH 4). When an antibiotic sensitivity test was performed on *E. faecium* HL7, this isolate was found to be resistant to trimethoprim/sulfamethoxazole, cephalothin, ampicillin, rifampin, gentamicin, cefotaxime, cefepime, cefotetan, nalidixic acid, and kanamycin.

NEW FUNCTIONAL FOODS WITH PROBIOTICS AND PREBIOTICS: PERSPECTIVES IN THE CONTROL OF OBESITY

Torriani, S.*

Department of Biotechnology, University of Verona, Verona, Italy

The health benefits provided by probiotics, prebiotics and synbiotics have been the subject of extensive research in the past few decades. These food supplements can be included in functional foods, and they can exert a positive impact on the human health, acting through the modulation of the intestinal microbiota, that strongly influences host physiology.

Particular disorders or pathologies (e.g. inflammatory bowel diseases, obesity, diabetes) can be related to dysbiosis conditions, that means changes in the composition and/or functions of the microbiota which cause an alteration of the healthy symbiosis with the host. Given the importance of the diet in the modulation of the gut microbiota, the use of functional foods as a strategy to shape the microbiota towards a "healthier" state could be a promising approach.

Among functional foods, the increasing market demand for non-dairy probiotic foods and beverages is orientating the research towards healthy alternatives to dairy-based products, useful for vegetarians and lactose intolerant customers. Therefore, the development of new functional products could combine the beneficial effects of vegetables, fruits or cereals with health promoting ingredients, including non-conventional probiotic species (such as *Bacillus* spp., *Escherichia coli*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*).

This was the approach followed in the development of a new functional pasta from a durum wheat flour rich in polyphenols, with added barley β -glucans and a probiotic spore-forming bacterium, *Bacillus coagulans* GBI-30, 6086 (Fares C. et al., 2015 J. Cereal Sci.; PASS-WORLD project). This functional pasta was given to overweight and obese subjects, leading to encouraging results such as the improvement of some inflammatory parameters and gut microbial balance.

Demonstration of clinical benefits, supported by knowledge on the mechanistic actions on the microbiota of the target population, remains a fundamental issue in the research area of functional foods in the control of pathological conditions, and it could be realistically achieved with a multidisciplinary approach, involving complementary technological, nutritional, clinical and microbiological competences.



1300	POSTER SESSION II: CLOSTRIDIUM DIFFICILE: PATHOGENESIS	
PII-32	The Role of Flagella in <i>Clostridium difficile</i> Pathogenesis: Comparison Between a Non-Epidemic and an Epidemic Strain <i>Baban, S.T.;* Kuehne, S.A.; Barketi-Klai, A.; Cartman, S.T.; Kelly, M.L.; Hardie, K.R.; Kansau, I.; Collignon, A.; Minton, N.P.</i>	175
PII-33	Assessment of Additional Virulence Factors Present in the <i>in vitro</i> Supernatant of <i>Clostridium difficile</i>	176
	Castro-Peña, C.;* López-Ureña, D.; Rodríguez, C.; Quesada-Gómez, C.; Chaves-Olarte, E.	
PII-34	Correlation of Binary Toxin with Clinical Outcomes in <i>C. Difficile</i> Infection (CDI)	177
	Cihlar S.;* Siddiqui, F.; Cheknis, A.; Sambol, S.P.; Carman, R.; Lyerly, M.; Gerding, D.N.; Johnson, S.	
PII-35	Mucin-Associated Bacterial Communities During Clostridium difficile Infection	178
	Semenyuk, E.G.; Ashraf, A.; Poroyko, V.A.; Johnston, P.F.; Knight, K.L.; Gerding, D.N.; Driks, A.*	
PII-36	A Novel Negative Regulator of Sporulation Initiation in Clostridium difficile	179
	Edwards, A.N.;* Childress, K.O.; McBride, S.M.	
PII-37	Antigenic Differentiation of Toxigenic and Non-Toxigenic Strains of <i>Clostridium difficile</i>	180
	Gowrishankar, R.;* Williamson, Y.M.; Kirkham, H.; Barr, J.R. Moura, H.	
PII-38	Structural Studies of the <i>Clostridium difficile</i> toxin TcdA in Complex with a Neutralizing Monoclonal Antibody <i>Kroh, H.K.;* Chandrasekaran, R.; Ohi, M.D.; Nyborg, A.C.; Rainey, J.; Warrener, P.; Spiller, B.W.; Lacy, D.B.</i>	181
PII-39	Extracellular Vesicles of Clostridium difficile	182
	Lopes, A.S.; Silva, R.C.; Boente, R.F.; Domingues, R.D.P.; Miranda, K.R.; Lobo, L.A.*1	
PII-40	Proteomic Analysis of the Exosporium (Spores) of Brazilian Clostidium difficile Ribotypes Treated with Hospital Antibiotics	183
	Motta, K.O.L.S.; Trindade, C.N.R.; Ferreira, T.G.; Miyajima, F.; Domingues, R.M.C.P.; Ferreira, E.O.*	
PII-41	Metaproteomics Unveils the Fate of <i>Clostridium difficile</i> Toxins in Stool Samples	184
	Moura, H.;* Kraft, C.S.; Williamson, Y.M.; Kirkham, H.; Gowrishankar, R.; Barr, J.R.	

Clostridium difficile: Pathogenesis

Wednesday, July 13, 2016

Posters will be presented in Poster Session II Wednesday, July 13 1300-1400.

Wednesday, July 13, 2016 Clostridium difficile: Pathogenesis

PII-42	Exploring the Role of an Alanine Racemase during <i>Clostridium difficile</i> Spore Germination	185
	Shrestha, R.;* Sorg, J.A.	
PII-43	TPL-2 is a Key Regulator of Inflammation in C. difficile Infection	186
	Wang, Yu.; Ju, X.; Tzipori, S.; Feng, H.; Greenberg, A.; Sun, X.*	
PII-44	Inhibition of Spore Germination by Microbial Derived Secondary Bile Acids in <i>Clostridium difficle</i> Strains that Vary in Ribotype	187
	Thanissery, R.;* Theriot, C.M.	

Posters will be presented in Poster Session II Wednesday, July 13 1300-1400.

THE ROLE OF FLAGELLA IN CLOSTRIDIUM DIFFICILE PATHOGENESIS: COMPARISON BETWEEN A NON-EPIDEMIC AND AN EPIDEMIC STRAIN

Baban, S.T.;* Kuehne, S.A.; Barketi-Klai, A.; Cartman, S.T.; Kelly, M.L.; Hardie, K.R.; Kansau, I.; Collignon, A.; Minton, N.P. Clostridia Research Group, NIHR Biomedical Research Unit in GI Disease, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK

²Faculté de Pharmacie, EA4043, Université Paris-Sud, Châtenay-Malabry, France

Clostridium difficile is a major cause of healthcare-associated infection and inflicts a considerable financial burden on healthcare systems worldwide. Disease symptoms range from self-limiting diarrhoea to fatal pseudomembranous colitis. Whilst C. difficile has two major virulence factors, toxin A and B, it is generally accepted that other virulence components of the bacterium contribute to disease. C. difficile colonises the gut of humans and animals and hence the processes of adherence and colonisation are essential for disease onset. Previously, it has been suggested that flagella might be implicated in colonisation. Here, we tested this hypothesis by comparing flagellated parental strains to strains in which flagella genes were inactivated using ClosTron technology. Our focus was on a UK-outbreak, PCR-ribotype027(B1/NAP1) strain, R20291. We compared the flagellated wild-type to a mutant with a paralyzed flagellum and also to mutants (fliC, fliD, and flgE) that no longer produce flagella in vitro and in vivo. Our results with R20291 provide the first strong evidence that by disabling the motor of the flagellum, the structural components of the flagellum rather than active motility, is needed for adherence and colonisation of the intestinal epithelium during infection. Comparison to published data on $630\Delta erm$ and our own data on that strain revealed major differences between the strains: the R20291 flagellar mutants adhered less than the parental strain *in vitro*, whereas we saw the opposite in $630\Delta erm$. We also showed that flagella and motility are not needed for successful colonisation *in vivo* using strain 630Δ*erm*. Finally, we demonstrated that in strain R20291, flagella do play a role in colonisation and adherence and that there are striking differences between *C. difficile* strains. The latter emphasises the overriding need to characterize more than just one strain before drawing general conclusions concerning specific mechanisms of pathogenesis.

ASSESSMENT OF ADDITIONAL VIRULENCE FACTORS PRESENT IN THE *IN VITRO* SUPERNATANT OF CLOSTRIDIUM DIFFICILE

Castro-Peña, C.;* López-Ureña, D.; Rodríguez, C.; Quesada-Gómez, C.; Chaves-Olarte, E.

Centro de Investigación en Enfermedades Tropicales and Facultad de Microbiología. Universidad de Costa Rica, San José, Costa Rica

The epidemic NAP1/027 strain is responsible for *Clostridium difficile* nosocomial outbreaks worldwide. The increased pathogenic potential of this strain has been attributed to overproduction of toxins among other properties. During a *C. difficile* outbreak in Costa Rica, a new virulent genotype was identified and denominated NAP_{CR1}/RT012. Strains from this genotype do not overproduce toxins. However, bacteria-free supernatants from the NAP_{CR1}/RT012 strain induce a pathogenic response similar to NAP1/RT027 measured as neutrophil infiltration, edema and epithelial damage in the murine ligated ileal loop model. These results suggest the presence of factors other than the toxins in the supernatant that have an important role in the pathogenesis of the *C. difficile*-associated infections. To test this hypothesis, we intended to analyze the biological activities of toxin-depleted supernatants from NAP_{CR1} strain and compare it with corresponding supernatants from toxins hyper producer strains NAP1 and VPI10463. For this purpose, we grew the strains in a high-glucose medium, a condition known to repress toxin production. Using a combination of techniques, we proved the absence of toxins but a relatively unchanged proteomic profile. The induction of edema, epithelial damage, and immune activation induced by the toxin-depleted supernatants from the different strains, as well as by the corresponding supernatants supplemented with the same amount of purified Toxin A and Toxin B, will provide useful information regarding the presence of putative additional virulence factors. These factors might be responsible for the emergence of new virulent strains of this important nosocomial pathogen.

CORRELATION OF BINARY TOXIN WITH CLINICAL OUTCOMES IN *C. DIFFICILE* INFECTION (CDI)

Cihlar S.;*¹ Siddiqui, F.;^{1,2} Cheknis, A.;² Sambol, S.P.;^{1,2} Carman, R.;³ Lyerly, M.;³ Gerding, D.N.;^{1,2} Johnson, S.^{1,2}
¹Loyola University Medical Center, Chicago, IL USA
²Edward Hines, Jr. VA Hospital, Chicago, IL USA

³TechLab Inc., Blacksburg, VA USA

Evidence suggests that the severity, incidence, and complications of *Clostridium difficile* infection (CDI) are increasing. Recent data has implicated binary toxin CDT as a possible additional virulence factor in *C. difficile* strains associated with this recent increased CDI incidence. In order to look at the role of binary toxin in CDI, we tested stool specimens from patients with CDI for the presence of binary toxin using an enzyme immunoassay (EIA) that detects the binding portion of the toxin (CDTb), cultured the stools for *C. difficile*, typed the *C. difficile* isolates by restriction endonuclease analysis (REA), and performed PCR using *cdtB* primers for the receptor binding component of binary toxin. Clinical outcomes were correlated with detection of fecal binary toxin and with detection of a binary toxin-positive *C. difficile* strain.

93 consecutive patients with CDI from one Veterans Administration hospital had positive stool cultures for *C. difficile*. 10 of 86 (11.6%) evaluable patients had a subsequent CDI recurrence and 22 of 93 (23.6%) died within 60 days. 38 (40.9%) were infected with *C. difficile* isolates that were positive for binary toxin by PCR. The most common strain identified was the binary toxin-positive epidemic REA Group BI strain which accounted for 17 of 93 (18%) isolates. Eight additional binary-toxin positive strains were also identified. Six (60%) of the patients with a CDI recurrence were infected with binary toxin-positive strains compared to 29 (38%) patients who did not have a subsequent recurrence (P= 0.16). Eleven (50%) of the patients who died were infected with binary toxin-positive strains compared to 27 (38%) of patients who survived for at least 60 days (P= 0.23).14 (15%) of the patients had detectable fecal binary toxin by CDTb EIA. Nine of these patients (64%) were infected by the BI strain. Analysis of clinical outcomes in this subset was not significant.

In this study, CDI recurrence and mortality were more common in patients infected with binary toxin-positive *C. difficile* strains, but the difference did not reach statistical significance. Fecal binary toxin was detected in a subset of patients infected with binary toxin-positive strains, particularly those infected with the BI (aka 027) strains. Further analysis is being conducted to determine the influence of other potentially confounding factors, particularly prolonged treatment regimens and clinical management of patients in order to clarify the role of binary toxin in recurrent CDI and mortality.

MUCIN-ASSOCIATED BACTERIAL COMMUNITIES DURING CLOSTRIDIUM DIFFICILE INFECTION

Semenyuk, E.G.;¹ Ashraf, A.;¹ Poroyko, V.A.;² Johnston, P.F.;^{1,3} Knight, K.L.;¹ Gerding, D.N.;³ Driks, A.*¹

¹Department of Microbiology and Immunology, Loyola University Chicago, Stritch School of Medicine, Maywood, IL USA

²Department of Pediatrics, University of Chicago, Chicago, IL USA

³Hines Veterans Affairs Hospital, Hines, IL, USA

Clostridium difficile infection initiates with spore ingestion. Subsequently, the spore germinates and growing cells colonize the GI tract mucosa and cause disease. Little is known about the interactions of *C. difficile* with host mucosa or other GI tract bacteria during disease. To address this knowledge gap, we visualized *C. difficile* and other bacteria in the colon and cecum of infected mice at 1-8 days post infection (PI). We fixed GI tissue with Carnoy's fixative, embedded in paraffin, and performed fluorescent in situ hybridization (FISH), using appropriate 16S rRNA probes targeting various bacterial taxa as well as *C. difficile* specifically. We visualized *C.* difficile vegetative cells and spores in bacterial communities in the cecum and colon, starting at day 1 PI. Strikingly, the spores were encased in the vegetative cell surface layer protein SlpA. C. difficile was a minority member of these communities. Using an antibody specific to the mucin protein Muc2, we showed that these communities are associated with the loose, outer mucus layer. We also partially characterized the taxa present in these communities using FISH. To do this, we performed microbial community profiling using 16S rRNA gene sequencing of bacterial DNA prepared from intestinal contents. This allowed us to design additional probes for FISH, to identify specific bacterial taxa in the communities. Using these probes, we detected members of Bacteroidaceae, Enterobacteriaceae, Lactobacilaceae, and Enterococcaceace families, as well as the Clostridium coccoides-Eubacterium rectale group, in communities harboring C. difficile. These data suggest that formation of multi-species communities associated with the mucus of the cecum and colon is an important step in GI tract colonization. Our results also demonstrate that spores form within these mucosal communities.

A NOVEL NEGATIVE REGULATOR OF SPORULATION INITIATION IN CLOSTRIDIUM DIFFICILE

Edwards, A.N.;* Childress, K.O.; McBride, S.M. Department of Microbiology and Immunology, Emory University, Atlanta, GA USA

The formation of dormant spores is critical for survival outside of the host for the gastrointestinal pathogen, *Clostridium difficile*. Persistence of *C*. difficile spores in medical facilities greatly contributes to the spread of C. difficile infection (CDI), and spore resistance to anti-infectives allows for recurrent infections in individuals. Despite the importance of sporulation to C. difficile pathogenesis, the molecular mechanisms and environmental signals controlling spore formation are not well understood. The initiation of sporulation is regulated through the phosphorylation state of the highly conserved transcription factor, Spo0A. Multiple regulatory factors influence Spo0A phosphorylation in other spore-forming bacteria; however, many of these factors are not conserved in C. difficile, and few novel factors have been identified. Here, we investigate the function of a protein, CD1492, which is annotated as a kinase and originally proposed to promote sporulation by directly phosphorylating Spo0A. Surprisingly, a null mutation in this gene results in increased sporulation, suggesting that CD1492 is a negative regulator of C. difficile sporulation. We observed an increase in sporulationspecific gene expression in the CD1492 mutant, further confirming that CD1492 inhibits spore formation. Deletion of CD1492 also negatively impacted toxin production *in vitro*, which resulted in a less virulent phenotype in the hamster model of CDI. Further, the CD1492 mutant demonstrated multiple effects on gene transcription that are not associated with sporulation or Spo0A activity, indicating that this factor interacts with pathways other than those directly involved in sporulation. Altogether, these data indicate that CD1492 negatively controls C. difficile sporulation and positively influences toxin production and virulence. Our study reveals a novel regulatory pathway that controls *C. difficile* sporulation.

ANTIGENIC DIFFERENTIATION OF TOXIGENIC AND NON-TOXIGENIC STRAINS OF CLOSTRIDIUM DIFFICILE

Gowrishankar, R.;* Williamson, Y.M.; Kirkham, H.; Barr, J.R.; Moura, H. Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control, Atlanta, GA USA

Clostridium difficile (Cdiff) are spore-forming, anaerobic bacteria, and the etiologic agent of *C. difficile* infection. Cdiff are clinically significant as the pathogen is the leading cause of hospital-acquired diarrhea. The pathogenicity of Cdiff stems from the production of two potent secreted toxins; an enterotoxin (TcdA) and a cytotoxin (TcdB). Non-toxigenic Cdiff strains have been distinguished from toxigenic strains based on a combination of bio-molecular techniques. We report here a gel-based proteomic approach to characterize Cdiff toxigenic and non-toxigenic strains. Five ATCC Cdiff strains were grown at 24, 48, and 72 hours, cells harvested and the culture supernatants (CS) retained. First, CS proteins were denatured, separated by SDS-PAGE, followed by silver staining or transferred to a PVDF membrane for western blotting. Second, images of the separated protein gel bands/immunoblots were processed and comparatively analyzed using BioNumerics, a bioinformatics software. Gel analysis displayed common and differential banding patterns around 270 kDa and 307 kDa, masses typically associated with toxins, for all but one Cdiff strain. Additionally, cluster analysis by BioNumerics grouped the four toxigenic strains together, while the non-toxigenic strain was identified as an outlier. Alternatively, we applied electrospray-ionization mass spectrometry (ESI-MS) bottom-up proteomics to profile toxigenic and non-toxigenic Cdiff isolates. Both qualitative and quantitative differences in proteins associated with Cdiff virulence were observed. Around 200 CS proteins were identified with high confidence using ESI-MS. Although many of the identified proteins were present in both non-toxigenic and toxigenic strains, toxins TcdA and TcdB were clearly absent in the non-toxigenic strain. In summary, the use of gel electrophoresis and immunoblotting techniques in parallel with MS are effective approaches for distinguishing Cdiff toxigenic and nontoxigenic strains.

STRUCTURAL STUDIES OF THE CLOSTRIDIUM DIFFICILE TOXIN TCDA IN COMPLEX WITH A NEUTRALIZING MONOCLONAL ANTIBODY

Kroh, H.K.;*1 Chandrasekaran, R.;1 Ohi, M.D.;1 Nyborg, A.C.;2 Rainey, J.;2 Warrener, P.;2 Spiller, B.W.;1 Lacy, D.B.1

¹Vanderbilt University School of Medicine, Nashville, TN USA ²MedImmune LLC, Gaithersburg, MD, USA

Clostridium difficile is a clinically significant pathogen that causes mildto-severe (and often recurrent) infection in the colon. Symptoms stem from the activities of two large, multi-domain toxins known as TcdA and TcdB. The toxins can bind, enter, and perturb host cell function through a multi-step mechanism of receptor binding, endocytosis, pore formation, autoproteolysis, and glucosyltransferase-mediated modification of host substrates. Monoclonal antibodies that neutralize the activities of the toxins have been shown to provide a survival benefit in preclinical animal models and prevent recurrent infections in human clinical trials. We have performed structural studies on a neutralizing monoclonal antibody, PA50, in complex with TcdA. Electron microscopy imaging shows that PA50 binds multiple sites on the TcdA C-terminal CROPS domain. A crystal structure of two PA50 Fabs bound to a segment of the TcdA CROPs provides a view of the molecular determinants for this interaction and suggests a mechanism wherein antibody binding disrupts cell surface carbohydrate recognition. The PA50 antibody is promising as a new tool to characterize essential functional regions required for TcdA intoxication.

EXTRACELLULAR VESICLES OF CLOSTRIDIUM DIFFICILE

Lopes, A.S.;¹ Silva, R.C.;³ Boente, R.F.;³ Domingues, R.D.P.;¹ Miranda, K.R.;² Lobo, L.A.*¹

¹Campus Fundão, Universidade Federal do Rio de Janeiro, RJ Brazil
²Campus Macaé, Universidade Federal do Rio de Janeiro, Macaé, RJ Brazil
³Inmetro—Instituto Nacional de Metrologia Qualidade e Tecnologia[,] Rio de Janeiro, RJ Brazil

Some bacteria produce and release membrane vesicles (MV) originated in the cellular membranes. In Gram negative bacteria, these structures originate in the outer membrane (OMVs) and have been studied in detail. The OMVs carry several molecules for long distances, including LPS, toxins, DNA, and bacterial virulence factors. Although Gram positive does not possess an outer membrane, vesicles originated in the cytoplasmic membrane have been described. In *Clostridium difficile*, a Gram positive bacteria, producer of toxins and with high levels of antibiotic resistance, these structures have not been detected yet. This study investigates the existence of these vesicles in a NAP strain of *C. difficile*. The extraction and purification of the vesicle were performed by ultrafiltration of the supernatant of a stationary phase culture followed by differential centrifugation in a density gradient, the fractions obtained were analyzed by transmission electron microscopy (TEM). The results show the presence of high amounts of vesicles wit a diameter range from 20 to 400 nm. Polyacrylamide gel electrophoresis and silver staining was employed to verify the protein pattern of MV fractions. Commercial ELISA assays were unable to detect the presence of toxin A and B in the MV fractions. To our knowledge, this is the first study to point out presence of MVs in *C. difficile*. The identification of the proteins and cellular components segregated in MV will help us understand important steps in the pathogenesis of *C. difficile*. Production of vesicles and his productd might also be useful for vaccine developmens since previously studies utilized that structure as a possible inductor of the immune response.

Funding: FAPERJ, CNPq, CAPES

PROTEOMIC ANALYSIS OF THE EXOSPORIUM (SPORES) OF BRAZILIAN *CLOSTIDIUM DIFFICILE* RIBOTYPES TREATED WITH HOSPITAL ANTIBIOTICS

Motta, K.O.L.S.;¹ Trindade, C.N.R.;¹ Ferreira, T.G.;¹ Miyajima, F.;³ Domingues, R.M.C.P.;¹ Ferreira, E.O.*^{1,2} ¹IMPG—Universidade Federal do Rio de Janeiro, RJ Brazil ²UFRJ- Polo Xerém, RJ Brazil ³Medical Research Council University of Liverpool, Liverpool UK

Clostridium difficile (Cdiff) is the most common cause of nosocomial diarrhea. Since spore formation represents an important virulence factor for this pathogen, the main goal of this study was to identify proteins of the exosporium from two Brazilian Cdiff ribotypes (RT133 and RT135) and NAP1 (RT027) strain, for comparison, after the strains were exposed to sub inhibitory concentrations of clindamycin and levofloxacin. Antibiotics were added to 70:30 agar plates medium and those were incubated for 10 days at 37°C under anaerobic conditions. Colonies formed were scraped from plates and kept under refrigeration (4°C) for 24h. The sediment was resuspended in a lysis buffer at 37°C for 2h under agitation. The content was applied to a 50% sucrose gradient and centrifuged by using a *swinging-bucket* rotor. Spores were washed and lysate with a buffer (0.1M sodium borate pH 10; 0.5% SDS and 50mM DTT). Proteins were applied to a SDS-PAGE and bands excised to be processed for Maldi TOF/TOF MS. All spectra generated were compared to the NCBInr database (MASCOT). So far, we have identified 53 proteins from the RT135, and most of them are related to the Clostridium spp. and Bacillus spp. spores. Only 4 of these proteins identified were present in all three conditions. Cdiff spore proteins identified include a superoxide dismutase family and CotE, a bifunctional peroxiredoxin reductase and chitinase. Complementary analysis, by using a pool of sera from patients, followed by a Western-Blotting showed that specific spore proteins were recognized when cells were exposed to Clindamycin. Other experiments are been conducted to identify those proteins and find out their function and correlation in the sporulation process. As a preliminary conclusion, we would like to emphasize that antibiotics exposition can modify the expression of spore proteins, which have never been speculated. The identification of the spores proteins can help to elucidate *C. difficile* pathogenesis and comprehend the sporulation process.

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

METAPROTEOMICS UNVEILS THE FATE OF CLOSTRIDIUM DIFFICILE TOXINS IN STOOL SAMPLES

Moura, H.,*1 Kraft, C.S.;² Williamson, Y.M.;¹ Kirkham, H.;¹ Gowrishankar, R.;¹ Barr, J.R.¹

¹Division of Laboratory Sciences, NCEH, CDC, Atlanta, GA USA ²Pathology and Laboratory Medicine, Emory University Atlanta, GA USA

Laboratory confirmation of infections caused by Clostridium difficile (Cdiff) is a challenge. A fast, sensitive, and specific method for toxin detection and quantification is still lacking. Understanding the fate of Cdiff toxins in stool samples might advance and improve detection. Previously, we have studied Cdiff exoproteins using high-throughput mass spectrometry (MS)-based proteomics for the development of MS methods for Cdiff toxin detection and quantification. In this study, we applied metaproteomics and used in vitro digestion of Cdiff TcdA and TcdB with both trypsin and proteases from filtered stool supernatants. Our main initial finding was that Cdiff toxins are quickly digested in vitro and display various consistent digestion patterns. We ran a time-course digestion study of TcdA and TcdB combining western blot (WB) and MS analysis. WB revealed antigenic reactivity of toxin fragments with bands between 20-100 kDa and MS confirmed their specific peptides. We then conducted a pilot study using 36 consecutive Cdiff PCRpositive stool samples. Initially, we assessed the samples with TECHLAB Cdiff Quick Check® complete kit (CdQ), and we found that 25 (70%) were GDH positive and 21 (42%) were also toxin positive. Metaproteomic studies suggested that the majority of proteases found in the stool samples analyzed were from human origin and confirmed the absence of intact toxin in the stool samples. In addition, WB analysis revealed that antibodies specific to TcdA and TcdB reacted with bands that were analogous to those seen in the *in vitro* study. Interestingly, the same stool samples that only contained toxin fragments still rendered a positive CdQ result. Lastly, we were able to correlate toxin digested peptides found in stool samples with Cdiff biomarkers from in vitro experiments and determined that these toxin biomarkers could be found in 60% of the Cdiff PCR-positive stool samples. Better results were obtained after immunoprecipitation, which indicated that the toxin fragments could be concentrated by antibodies. The discovered biomarkers present in stool samples, if confirmed in a larger study, would pave the way for better diagnostic reagents to detect Cdiff toxins and other key products in stool samples.

EXPLORING THE ROLE OF AN ALANINE RACEMASE DURING CLOSTRIDIUM DIFFICILE SPORE GERMINATION

Shrestha, R.;* Sorg, J.A. Texas A&M University, College Station, TX USA

Antibiotic treatment is the greatest risk factor for *Clostridium difficile* infection, because antibiotics alter the normal ecology of the gut microbiota. Due to the strict anaerobic nature of the vegetative form, C. difficile survives outside a host as a dormant endospore. C. difficile spores are highly resistant to environmental stresses and are essential for transmission between hosts. Once in a host, the spores germinate to the vegetative, disease causing form. In vitro C. difficile spore germination is triggered by a combination of bile acids (e.g., taurocholic acid [TA]) and amino acids (e.g., glycine). L-alanine is also recognized as a co-germinant by C. difficile spores and L-alanine is the most common germinant among spore formers. L-alanine is predominant in most living organisms, and it is converted into D-alanine by alanine racemases (encoded by alr). In Bacillus subtilis, a model spore-forming organism, germination is triggered by L-alanine, and D-alanine inhibits L-alanine-mediated spore germination. Several spore-formers embed an alanine racemase in their spores. These alanine racemases are thought to convert L-alanine to D-alanine, thereby increasing the threshold required to activate spore germination in response to L-alanine. C. difficile also encodes an alanine racemase downstream of gerS (a newly identified lipoprotein that is important for *C. difficile* spore germination). Here, we investigate the importance of this racemase on *C. difficile* spore germination.

PII-43

TPL-2 IS A KEY REGULATOR OF INFLAMMATION IN C. DIFFICILE INFECTION

Wang, Y.;^{1,3} Ju, X.;¹ Tzipori, S.;¹ Feng, H.;⁴ Greenberg, A.;² Sun, X.*^{1,3}
¹Department of Infectious Disease and Global Health, Tufts University Cummings School of Veterinary Medicine, North Grafton, MA USA
²Obesity and Metabolism Laboratory JM-USDA Human Nutrition Research Center, Boston, MA USA

³Department of Molecular Medicine, Morsani School of Medicine, University of South Florida, Tampa, Fl USA

⁴Department of Microbial Pathogenesis, School of Dentistry, University of Maryland, Baltimore, MD USA

Tumor progression locus 2 (TPL-2) has a critical role in the response to inflammatory signals as it functions as a serine/threonine kinase in the MAPK signal transduction cascade known to regulate both innate and adaptive immunity. The pro-inflammatory actions of TPL-2 are mediated by the activation of MAPKs, including ERK, c-Jun NH₂-termianl kinase (JNK) and p38 MAPK. Both Clostridium difficile TcdA and TcdB are capable of inducing pro-inflammatory cytokines including TNF-α and IL-1β, which are implicated in the development and progression of *C. difficile* infection (CDI). Previously, we showed that both TcdA and TcdB could activate p38 MAPK and ERK in both Raw264.7 macrophages and bone marrowderived dendritic cells (BMDCs). We also found that TcdA-mediated TNF- α production in RAW264.7 cells was mediated through p38 MAP kinase and MEK/ERK signaling pathways. In this study, we investigated whether TPL2 has a central role in CDI severity by mediating the production of proinflammatory cytokines including TNF- α , IL-1 β , and IL-6. We report here that in BDMCs, a TPL2 specific inhibitor abolished TcdB-induced production of TNF-α, IL-1β, and IL-6. We further demonstrated that TPL2 inhibitor dramatically blocked TcdB-induced activation of ERK and p38 MAP kinase, but not of JNK in BMDCs. We confirmed these results using BMDCs extracted from TPL2-KO mice. Finally, we demonstrated TPL2-KO mice were significantly more resistant than wild-type mice to *C. difficile* infection in mice. Taken together, our data suggest TPL2 represents a potential therapeutic target for CDI treatment.

INHIBITION OF SPORE GERMINATION BY MICROBIAL DERIVED SECONDARY BILE ACIDS IN *CLOSTRIDIUM* DIFFICLE STRAINS THAT VARY IN RIBOTYPE

Thanissery, R.S.;* Theriot, C.M. Department of Population Health and Pathobiology, NCSU College of Veterinary Medicine, Raleigh, NC USA

The changing epidemiology of *Clostridium difficle* infection (CDI) over the past decades presents a significant challenge in the management of C. difficle associated diseases. Spore germination is a critical step of the *C. difficile* life cycle that leads to vegetative cell outgrowth and toxin production, which mediates disease. The gastrointestinal tract microbiota provides colonization resistance against C. difficile and growing evidence suggests that microbial derived secondary bile acids play a role. However, little is known about how secondary bile acids alter the *C. difficile* lifecycle, including spore germination, growth, and toxin production, in historic and more current strains that vary in ribotype. C. difficile strains that vary in age and ribotype, R20291 and CD196 (027), M68 (017), and M120 (078) were used to measure taurocholate (TCA) mediated spore germination and inhibition with microbial derived secondary bile acids (ωMCA, HDCA, UDCA, LCA, iLCA, DCA, iDCA) found in the mouse and human large intestine. Spores were incubated anaerobically with 0.1% TCA in brain heart infusion (BHI) broth with and without secondary bile acids for 30 min, at the end of which each sample was plated on to BHI media and BHI supplemented with 0.1% TCA to determine the level of spore germination. Spore germination of C. difficile ribotype 027 and 017 was significantly inhibited by all secondary bile acids, whereas ribotype 078 showed significantly less inhibition. Interestingly, the historic strain CD196 (027) was more sensitive to secondary bile acids at lower concentrations when compared to the more recent epidemic strain R20291 (027). C. difficile M120 (078) is a highly divergent strain resulting in minimal inhibition by most secondary bile acids with the exception of the highest concentrations of LCA (0.01%), iLCA (0.1%), and DCA (0.2%). Many secondary bile acids were able to inhibit TCA-mediated spore germination in a dose dependent manner, but the level of inhibition varied by strain and ribotype. Future studies will investigate how secondary bile acids alter other steps of the *C. difficile* life cycle.



Thursda	ay, July 14, 2016 Clostridium difficile: Epidem	iology
1320	POSTER SESSION III: CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY	
PIII-1	Ribotype Diversity of Recent Clinical Clostridium difficile Strains Circulating in and around Houston, Texas Alam, M.J.;* McPherson, J.; Miranda, J.; Amadio, J.; Kuo, J.; Garey, K.W.	191
PIII-2	High Colonization of <i>Clostridium difficile</i> among Different Nigerian Age Groups; Possible Reason for Low Incidence of CI <i>Egwuatu, T.O.;</i> * <i>Ogunsola, F.T.; Olalekan, A.O.; Egwuatu, C.A</i>	
PIII-3	Emergence of a New <i>C. difficile</i> Strain, REA Group DQ, Related to REA Group BI/Ribotype 027 Johnson, S.;* Petrella, L.; Siddiqui, F.; Sambol S.P.; Gulvik, C.; Limbago, B.; Gerding, D.N.; Donskey, C.J.	193
PIII-4	Molecular Epidemiology of <i>Clostridium difficile</i> Isolated in the United States, 2014 <i>Karlsson, M.;* Paulick, A.; Albrecht, V.; Granade, M.; Guh, A.; Rasheed, J.K.</i>	194
PIII-5	Clostridium difficile Infection Surveillance in a Tertiary Medical Center Reveals Significant Strain Variation Mansoor, A.;* Shehab, K.; Anwar, F.; Viswanathan, V.K.; Vedantam, G.	195
PIII-6	Occurrence of <i>Clostridium difficile</i> Infections in Hospitals of Silesia, Poland Aptekorz, M.; Szczegielniak, A.; Harmanus, C.; Kuijper, E.; Martirosian, G.*	196
PIII-7	The Role of Ribotype 106 as a Cause of Clostridium difficile Infection in the United States, 2012-2014 Paulick, A.;* Karlsson, M.; Albrecht, V.; Granade, M.; Guh, A.; Rasheed, J.K.; Limbago, B.; EIP CDI Pathogen Group	197
PIII-8	Identification and Characterization of <i>Clostridium difficile</i> Strains Isolated from Dog Stools in Rio De Janeiro, Brazil <i>Rainha, K.; Fernandes, R.F.; Miyajima, F.; Roberts, P.; Santos, J.; Domingues, R.M.C.P.; Ferreira, E.O.*</i>	198
PIII-9	Clostridium difficile Ribotype 027 is an Independent Risk Factor for Recurrent C. difficile Infection Rao, K.;* Young, V.B.	199
PIII-10	Characterization of <i>Clostridium difficile</i> Strains Isolated from Immunocompromised Patients in Brazil Secco, D.A.; Boente, R.F.; Miranda, K.R.; Santos-Filho, J.; Miyajima, F.; Nouer, S.A.; Domingues, R.M.C.P.*	200

Posters will be presented in Poster Session III Thursday, July 14 1320-1420.

Thurssday, July 14, 2016 Clostridium difficile: Epidemiology

PIII-11	Canine Pets are a Potential Source of Community Acquired Clostridium difficile Infection in Humans	201
	Stone, N.E.;* Sidak-Loftis, L.C.; Sahl, J.W.; Vazquez, A.J.; Busch, J.D.; Keim, P.; Wagner, D.M.	
PIII-12	Ribotype 017 among Toxigenic Isolates are Predominant Toxigenic <i>C. difficile</i> in Southern Taiwan <i>Hung, Y.P.; Tsai, B.Y.; Ko, W.C.; Tsai, P.J.</i> *	202
PIII-13	Colonization of Toxigenic <i>Clostridium difficile</i> among ICU Patients: A Prospective Study Zhang, X.; Wang, X.; Yang, J.; Zong, Z.*	203

RIBOTYPE DIVERSITY OF RECENT CLINICAL CLOSTRIDIUM DIFFICILE STRAINS CIRCULATING IN AND AROUND HOUSTON, TEXAS

Alam, M.J.;* McPherson, J.; Miranda, J.; Amadio, J.; Kuo, J.; Garey, K.W. University of Houston College of Pharmacy, Houston, TX USA

Background: Clostridium difficile is the most common cause of hospital acquired infectious gastroenteritis in the United States. Diverse and genotypically distinct spores of *C. difficile* from human and animal fecal contamination can persist in the environment and transmit to new patients. Our objectives of this study were to investigate the ribotype diversity of *C. difficile* isolates, and their molecular characteristics from two large hospital systems in Houston, Texas.

Methods: We isolated 609 *C. difficile* strains from 1559 CDI stool samples collected in 2014-15. Stool samples (~10-100mg) from patients that tested positive for *C. difficile* toxins were directly cultured onto CCFA plate and incubated at 37°C in an anaerobic chamber for 48h. Suspected isolates were then identified and characterized by PCR (*tcdA*, *tcdB*, *tpi*, *ctdA* and *ctdB* genes) and ribotyped using fluorescent PCR ribotyping technique.

Results: Twenty eight different ribotypes of *C. difficile* were found among 609 strains. The most common strain was ribotype 027 (21.2%; 129/609) followed by ribotypes 014-020 (16.4%), 002 (10.8%), UM11 (10.5%), 078-126 (7.1%), and UM8 (5.1%). Binary toxin (cdtA and cdtB) genes were present only in 027 and 078-126 strains.

Conclusions: Overall, hospitalized patients were infected with genotypically diverse toxigenic *C. difficile* strains. Long term surveillance on CDI patients is needed to prevent and control the transmission of *C. difficile* in hospitalized patient populations.

PIII-2 PIII-3

HIGH COLONIZATION OF *CLOSTRIDIUM DIFFICILE*AMONG DIFFERENT NIGERIAN AGE GROUPS; POSSIBLE REASON FOR LOW INCIDENCE OF CDI

Egwuatu, T.O.;*¹ Ogunsola, F.T.;² Olalekan, A.O.;³ Egwuatu, C.A.⁴¹Department of Microbiology, College of Medicine University of Lagos, Logos, Nigeria

²Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Lagos, Nigeris

³Medical Laboratory Science Department, College of Medicine, University of Lagos, Lagos, Nigeria

⁴Lifegate Specialist Hospital, Ikeja, Lagos, Nigeria

The past two decades have consistently seen the literature flooded with high incidences of Clostridium difficile, mostly from the Western region with little or nothing from the developing world. A study was, therefore, carried out to ascertain the prevalence of this organism in Lagos, Nigeria in order to establish its epidemiological and public health importance. A total of 763 faecal samples obtained from patients from primary health care centres, private hospitals, old people's home, patent medicine dealers, and laboratories were surveyed for the presence of C. difficile and its toxin in four randomly selected local Governments in Lagos metropolis, Nigeria. C difficile isolates and its toxins were identified using basic microbiological standards. A total of 156 C. difficile isolates were isolated from 763 patients surveyed in this study accounting for overall carriage rate of 20.4%. The highest carriage rate was seen in the ages ≥60 years (35.2%) and <1 year old (26.6%) and lowest rate in the age range of 10-19 (4.1%), while no isolate was obtained from new-borns. The major risk factor associated with the acquisition of C. difficile in this study was antibiotics usage in which amoxicillin, ampicillin and cephalosporin were mostly implicated. This study showed a high colonization rate of *C. difficile* among asymptomatic patients in different age groups suggesting the possibility of being protected from developing *C*. difficile infection hence, the reason for the low incidence of CDI and little or no reports of CDI from Nigeria.

EMERGENCE OF A NEW C. DIFFICILE STRAIN, REA GROUP DQ, RELATED TO REA GROUP BI/RIBOTYPE 027

Johnson, S.;*1.2 Petrella, L.;¹ Siddiqui, F.;¹.2 Sambol S.P.;¹.2 Gulvik, C.;³ Limbago, B.;³ Gerding, D.N.;¹.2 Donskey, C.J.⁴.5 ¹Hines VA Hospital, Chicago, IL USA ²Loyola University Medical Center, Chicago, IL USA ³Centers for Disease Control, Atlanta, GA USA ⁴Louis Stokes VA Hospital, Cleveland, OH USA ⁵Case Western Reserve University, Cleveland, OH USA

As the continuing epidemic of *C. difficile* infections due to PCR ribotype [RT] 027/ restriction endonuclease analysis [REA] Group BI is now in its second decade, new, related strains have emerged. RT244 strains in Australia and RT176 strains in Eastern Europe have similar, but distinct ribotype patterns and are located in the same Clade as 027 by MLST and whole genome sequencing (WGS). We describe a new *C. difficile* strain that was part of an outbreak at a long-term care facility (LTCF) and affiliated acute care facility in Ohio. This strain was initially identified as RT027 by a clinical laboratory using a commercial PCR test on patient stool specimens (Xpert *C. difficile* PCR assay, Cepheid, CA). Culture and typing of the *C. difficile* isolates by REA revealed a strain distinct from BI (RT027). We performed additional characterization to determine strain relatedness to BI, including toxinotyping, PCR amplification of binary toxin genes, sequencing of the *tcdC* regulatory gene, PCR ribotyping, and WGS.

HindIII REA typing of *C. difficile* isolates from 7 patients in the LTCF and 11 patients on acute care wards revealed identical REA patterns (REA Type DQ1). RFLP-PCR of the B1 and A3 fragments of the pathogenicity locus from a representative DQ isolate identified it as toxinotype III. Binary toxin was identified by PCR, and sequencing of *tcdC* identified an 18bp deletion and single base deletion at nt117, characteristics common to the BI/ RT027 strain, but the ribotype pattern was identified as RT591. WGS analysis indicated distinct clustering of these two strains. Both strains belong to MLST clade 2, but DQ/ RT591 are characterized as ST 41, whereas BI/ 027 are ST1. REA Group DQ/ RT591 is a newly identified *C. difficile* strain related to BI/ RT027 by toxinotyping, binary toxin and *tcdC* analysis, but distinct by REA type, PCR ribotype, and WGS. Surveillance for this strain is warranted given the epidemic nature, increased clinical severity and worse outcomes frequently associated with RT027 and related strains.

MOLECULAR EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE* ISOLATED IN THE UNITED STATES, 2014

Karlsson, M.;* Paulick, A.; Albrecht, V.; Granade, M.; Guh, A.; Rasheed, J.K.; Limbago B.; EIP CDI Pathogen Group Centers for Disease Control and Prevention, Atlanta, GA USA

Clostridium difficile, which is the leading cause of diarrhea and colitis in healthcare facilities, is now emerging in community settings. To better understand the molecular epidemiology of *C. difficile* infection (CDI) in the United States and how it might be changing, we performed molecular characterization of *C. difficile* isolates collected through the Emerging Infections Program (EIP) at the Centers for Disease Control and Prevention (CDC). During 2014, CDI surveillance was conducted in 10 EIP sites (CA, CO, CT, GA, MD, MN, NM, NY, OR, and TN). A convenience sample of clinical laboratories across the EIP sites submitted *C. difficile*—positive stool specimens from cases with full medical-record review to the MN Public Health State Laboratory and the Hines VA Hospital for culture, and isolates were forwarded to CDC. Each isolate was characterized by capillary-based PCR-ribotyping and PCR detection of *tcdA*, *tcdB*, *cdtA*, *cdtB*, and deletions in *tcdC*.

Among 1,123 isolates submitted in 2014, 134 different ribotypes (RTs) were detected. The predominant RT was 106, followed by 027, 002, and 020. The majority of isolates (95%) were tcdA- and tcdB-positive, and 247 (22%) were binary toxin-positive. The most common binary toxin-positive RTs were 027 and 078. Among 619 isolates from community-associated (CA) CDI, RT106 (11%) predominated followed by 002 (8%) and 020 (8%). Among 504 healthcare-associated (HA) cases, RT027 (14%) was most common. Although 027 was the most common RT among HA CDI, RT027 decreased from 24% in 2013 to 14% in 2014 among HA CDI, and from 12% to 7% among CA CDI. A notable decrease was observed in California, where RT027 constituted 26% of their HA submissions in 2013 but only 6% in 2014. Similarly, RT027 constituted 11% of the CA submissions in California in 2013, whereas no RT027 were submitted among CA CDI in 2014. This evaluation of isolates confirms that RT106 has surpassed RT027 as the most frequently identified RT. Continued surveillance and molecular typing of *C. difficile* isolates remain important to identify epidemic and emerging strains.

CLOSTRIDIUM DIFFICILE INFECTION SURVEILLANCE IN A TERTIARY MEDICAL CENTER REVEALS SIGNIFICANT STRAIN VARIATION

Mansoor, A.;*1 Shehab, K.;2 Anwar, F.;1 Viswanathan, V.K.;1 Vedantam, G.1 School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ USA

²College of Medicine, University of Arizona, Tucson, AZ USA

Clostridium difficile infection (CDI) is typically precipitated by antibiotic-mediated clearance of gut microbiota. While community-associated CDIs have been reported, few studies have parsed between hospital-acquired and hospital-precipitated CDI. Currently, there are no clear molecular 'signatures' that distinguish hospital-acquired from community-associated strains.

Objective: To monitor *Clostridium difficile* (CD) frequency, and compare isolates from patients, as well as those recovered from the hospital environment.

Methods: CD prevalence was monitored in a 487-bed hospital during two 3-month periods in Years 2011 and 2015. In 2015, only Intensive Care Units (ICUs) were monitored, and both patients and hospital surfaces targeted. For patient specimens, CD was cultured from diarrheic stool, and further characterized via ribotype analysis. For abiotic surfaces, wards, and common areas in three ICUs were swabbed, and swab-broth cultured for CD. Unique isolates were also assessed for toxin production. Comparative proteomics was performed on a subset of patient isolates using fully-quantitative mass spectrometry.

Results: A total of 53 (in 2015) and 21 (in 2011) unique CD isolates respectively, were obtained from patients, and belonged to a wide range of ribotypes (>30). However, the 2015 study revealed an increase in the "outbreak-associated" RT027 ribotype. CD isolates—even those of identical ribotype—expressed a wide range of toxin levels, suggesting that they were not clonal. Of 150 ICU surface swabs, CD was isolated from only two localized areas, and was of RT027. Proteomic analyses suggested broad differences even within strains belonging to the same ribotype, and a possible community-associated 'signature' was not readily identifiable.

Conclusions: The distribution of CD ribotypes in patient isolates suggests that patients may acquire CD in the community, but that CDI itself is likely hospital-precipitated. Prospective monitoring of all patients could therefore be effective in defining the burden of community-acquired CDI.

OCCURRENCE OF *CLOSTRIDIUM DIFFICILE* INFECTIONS IN HOSPITALS OF SILESIA, POLAND

Aptekorz, M.;¹ Szczegielniak, A.;¹ Harmanus, C.;² Kuijper, E.;² Martirosian, G.*¹

¹Department of Medical Microbiology School of Medicine in Katowice Medical University of Silesia, Poland

²Department of Microbiology, Leiden University Medical Center, Leiden, The Netherlands

The purpose of this study was to characterize *C. difficile* infections among patients in general hospitals of Silesia, Poland (wards of internal medicine, general surgery, hematology, cardiology, gastroenterology, anesthesiology, nephrology, pulmonology, urology, neurosurgery, gynecology/obstetrics, outpatients clinic, and others). Diarrhea was defined on the basis of generally accepted principles (≥ 3 loose stools in 24 hours).

CDI was diagnosed by using 2/3 steps diagnostic algorithm. Stool samples obtained from adult patients with diarrhea in 2013-2015 were tested for GDH by CDIFF QUICK CHEK COMPLETE, toxin A/B by ELISA (TechLab, USA), by molecular IllumiGene test (Meridian Bioscience, USA) and were cultured anaerobically (A35 Whitley anaerobic Workstation, UK). Isolated strains were identified using VITEK 2 Compact (bioMerieux, Marcy L'Etoile, France) and ribotyped. Antibiotic susceptibility (MIC for 11 antibiotics) was tested with E-tests.

Nitroimidazoles and fluoroquinilones were the often used antibiotics before abdominal symptoms were started. More frequent isolated ribotype of *C. difficile* was hyperepidemic PCR 027 (82.7%). These strains were multiresistant to 3 or more antibiotics.

Our study confirmed that the used algorithm allows to rapid and correct diagnosis of CDI. Demonstration of toxigenic *C. difficile* strains in majority of patients with symptoms of infection promotes the use of such a diagnostic algorithm for routine diagnostic of CDI. A high incidence of *C. difficile* PCR 027 ribotype resistant to 3 or more antibiotics was demonstrated in southern Poland.

The role of detected inflammatory factors (fecal lactoferrin and calprotectin), selected mechanisms of antibiotic resistance and demographic factors will be discussed.

This study was parcially financed by the "Diamond Grant" No. 207280 from Polish Ministry of Science and Higher Education and Grants of Medical University of Silesia No. KNW-1-086/K/5/0 and No. KWN-2-030/D/4/N.

THE ROLE OF RIBOTYPE 106 AS A CAUSE OF *CLOSTRIDIUM DIFFICILE* INFECTION IN THE UNITED STATES, 2012-2014

Paulick, A.;* Karlsson, M.; Albrecht, V.; Granade, M.; Guh, A.; Rasheed, J.K.; Limbago, B.; EIP CDI Pathogen Group Centers for Disease Control and Prevention, Atlanta, GA USA

Clostridium difficile ribotype (RT) 106, an epidemic strain in the United Kingdom, characteristically produces toxins TcdA and TcdB, is binary toxinnegative, and belongs to North American Pulsed-field type 11 (NAP11). In the United States, *C. difficile* isolates have been collected through the Emerging Infections Program (EIP) at the Centers for Disease Control and Prevention (CDC) since 2009. In 2014, RT106 became the most prevalent RT detected among EIP submissions. Here, we describe the prevalence and epidemiologic characteristics of RT106 isolates from the United States, 2012-2014.

Between 2012-2014, *C. difficile* infection (CDI) surveillance was conducted in 10 EIP sites (CA, CO, CT, GA, MD, MN, NM, NY, OR, TN). Isolates cultured from a subset of *C. difficile* cases were forwarded to CDC and characterized by capillary-based PCR-ribotyping and PCR detection of *tcdA*, *tcdB*, *cdtA*, *cdtB*, and deletions in *tcdC*.

Among 3,794 isolates submitted in 2012-2014, 366 (10%) were RT106, of which 96% were *tcdA*- and *tcdB*- positive, binary toxin-negative, with a wildtype *tcdC* sequence. The prevalence of RT106, stratified by community-associated (CA) and healthcare-associated (HA) CDI, respectively, was 9% and 9% in 2012, 9% and 8% in 2013, and 11% and 12% in 2014. During this period, an increase in RT106 was observed among HA CDI and CA CDI in 6 and 4 EIP sites, respectively. The largest increase was seen among HA CDI in California (6% to 23%). The most notable decrease in RT106 from 2012 to 2014 was observed among HA CDI submissions from CT (15% to 4%). In 2014, RT106 was the most prominent RT identified among CA cases in 4 sites (GA, MD, NY, and TN) whereas RT106 predominated among HA CDI in 3 sites (CA, CO, and MN). An outbreak with RT106 as the predominant epidemic strain was recently reported in the United States. This further highlights the importance of continued surveillance to characterize ongoing changes in the molecular epidemiology of *C. difficile*.

PIII-8 PIII-9

IDENTIFICATION AND CHARACTERIZATION OF CLOSTRIDIUM DIFFICILE STRAINS ISOLATED FROM DOG STOOLS IN RIO DE JANEIRO, BRAZIL

Rainha, K.;¹ Fernandes, R.F.;² Miyajima, F.;⁵ Roberts, P.;⁵ Santos, J.;³ Domingues, R.M.C.P.;³ Ferreira, E.O.*³,⁴

¹Universidade Federal Fluminense, Nova Friburgo, Curso de Biomedicina, Nova Friburgo, RJ Brazil

²Clínica Veterinária VetCare, Rio de Janeiro, Brazil

³Universidade Federal do Rio de Janeiro, IMPG, Lab. de Biologia de Anaeróbios, Rio de Janeiro, Brazil

⁴UFRJ- Polo Xerém, Duque de Caxias, RJ Brazil

⁵Universidade de Liverpool, Royal Hospital, Liverpool UK

Clostridium difficile (Cdiff) is the etiological agent of nosocomial bacterial diarrhea and the use of antibiotics predisposes susceptible patients to an imbalance of microbiota and colonization. Two cytotoxins (TcdA and TcdB) released by pathogenic strains are the cause of Clostridium difficile Infection (CDI). Cdiff gastrointestinal infections in dogs are still poorly elucidated and the possibility of transmission to humans being suggested. Thus, the aim of this study was to isolate Cdiff strains of dog stools, healthy or with diarrhea, in Rio de Janeiro. Thirty samples, among these 10 diarrheal, were randomly selected without distinction of sex or race, and kept frozen (-20°C) until the moment of use. For the early identification, after an alcoholic shock, the samples were inoculated into BHI broth (0.1% sodium taurocholate- ST) and kept at 37°C under anaerobic conditions for at least 72h. After this period, an inoculum was made in a BHI-agar (0.0128 mg/L D-cycloserine; 500 mg/L cefoxitin and 0.1% ST) and the plates were incubated anaerobically for 48h at 37°C. All colonies characterized as Cdiff had their identification confirmed by biochemical tests, the Cdiff Quick chek kit and Maldi-TOF (Bruker). A PCR species-specific using olygos for the gene *tpi* (triose phosphate isomerase) and toxins (tcdA and tcdB) were also performed. Of all samples, 5 (10%) were confirmed as Cdiff and two were toxigenic (PCR and Cdiff Quick complete). A susceptibility test also showed they were resistant to clindamycin. Most isolates were from animals with diarrhea and age ranging from 2 months to 5 years. Ribotyping and a biofilm assay are being conducted. We believe that this study will contribute to the data for the Cdiff strains, their zoonotic potential, and aid in the treatment of enteric diarrhea in animals.

Financial support: Capes, CNPq and FAPERJ

CLOSTRIDIUM DIFFICILE RIBOTYPE 027 IS AN INDEPENDENT RISK FACTOR FOR RECURRENT C. DIFFICILE INFECTION

Rao, K.;* Young, V.B. University of Michigan, Ann Arbor, MI USA

Background: Recurrent *C. difficile* infection (rCDI) frequently complicates recovery from CDI. Although treatments such as the antibiotic fidaxomicin, fecal transplant, and monoclonal antibodies can reduce the risk of rCDI, use is limited by cost and logistics. Accurately predicting rCDI would allow judicious allocation of limited resources. Published models have met with limited success. This study tested whether polymerase chain reaction (PCR) ribotype independently predicted rCDI.

Methods: Stool from non-pregnant inpatients of age≥18 years with diarrhea were included from 10/2010–1/2013 after testing positive for *C. difficile* in the clinical microbiology laboratory by enzyme immunoassay (EIA) for glutamate dehydrogenase and either a positive EIA for toxin(s) A/B or positive PCR for the *tcdB* gene. Chart review extracted clinical data. Per guidelines, rCDI was defined as a positive test for *C. difficile* >2 weeks but ≤8 weeks from the index episode. Anaerobic culture was used to grow and isolate *C. difficile*. All isolates were confirmed to be toxigenic *C. difficile* via PCR with *C. difficile*-specific primers for taxonomy and the *tcdA/tcdB* genes. All isolates were ribotyped. Simple and multiple logistic regression was used to model the primary outcome of rCDI.

Results: In total, 927 patients with 968 index episodes of CDI were included, with 110 (11.4%) developing rCDI. Mean age was 57.1 (standard deviation 3.2). There were 526 females (54.3%); 72.5% of the CDI episodes were healthcare-associated (onset >48 after admission); 36.6% had detectable stool toxins by EIA, and 13.7% of isolates were from ribotype 027. Age and use of proton pump inhibitors / concurrent antibiotics did not increase the risk of rCDI. Serum bilirubin level, detectable stool toxins by EIA, and ribotype 027 were associated with rCDI on unadjusted analysis, with healthcare-associated CDI being inversely associated. In the final multivariable model that included variables significant on unadjusted analysis, ribotype 027 was the strongest independent predictor of rCDI (odds ratio 1.89, 95% confidence interval 1.15–3.11, P = .013).

Conclusion: Ribotype 027 is an independent predictor of rCDI. Knowledge of the infecting *C. difficile* strain may aid clinical decisionmaking.

PIII-10 PIII-11

CHARACTERIZATION OF CLOSTRIDIUM DIFFICILE STRAINS ISOLATED FROM IMMUNOCOMPROMISED PATIENTS IN BRAZIL

Secco, D.A.; Boente, R.F.; Miranda, K.R.; Santos-Filho, J.; Miyajima, F.; Nouer, S.A.; Domingues, R.M.C.P.*

¹Universidade Federal do Rio de Janeiro—UFRJ, Rio de Janeiro, Brazil

²Faculdade de Farmácia, UFRJ, Macaé, RJ Brazil

³Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool UK

⁴Hospital Infection Control Commision, UFRJ, RJ Brazil

Clostridium difficile is a Gram-positive anaerobic bacterium, often associated with nosocomial diarrhea. The establishment of this organism in the colon results from the removal of members of the normal intestinal flora by antibiotic therapy. The main virulence factors associated with pathogenic strains are toxins A and B, and some strains produce binary toxin. This study aimed to isolate and characterize strains of *C. difficile* from patients admitted to the Hematology, Oncology and Transplant wards at Hospital Universitário Clementino Fraga Filho). Between 2013 and 2014, stool samples from 12 patients were collected. For immunological detection of toxins A and B, a ELISA kit was used. All fecal samples were subjected to alcohol shock and plated on CCFA medium for isolation of *C. difficile*. Three samples, two from the same patient, were positive for the presence of toxins A/B by ELISA, and three strains were isolated. Identification was confirmed by PCR for the specie-specific gene, tpi. PCR was also used to test for A, B, and binary toxins genes. The strains isolated from the same patient were positive for toxins A and B genes and belonged to ribotype 014/020. The remaining strain was shown to carry A, B and binary toxins genes. Sequencing showed mutations at the negative regulatory gene tcdC. This isolate belongs to a new ribotype not labeled yet. The antibiotic susceptibility was determined using E-test® strips. The strains were sensitive to metronidazole, vancomycin, teicoplanin, clindamycin, and moxifloxacin and resistant to levofloxacin. The strain belonging to a new ribotype showed a stronger cytotoxic effect in Vero cells, even if compared to NAP-1 strain. This is the first time that a binary toxin producer strain with *tcdC* deletion is detected in Brazil. Given the importance of this pathogen, the continuous monitoring of C. difficile and genetic/phenotypic characterization are aspects that deserve attention in clinical laboratories.

Financial support: CAPES, CNPq, FAPERJ

CANINE PETS ARE A POTENTIAL SOURCE OF COMMUNITY ACQUIRED CLOSTRIDIUM DIFFICILE INFECTION IN HUMANS

Stone, N.E.,* Sidak-Loftis, L.C.; Sahl, J.W.; Vazquez, A.J.; Busch, J.D.; Keim, P.; Wagner, D.M.¹ Northern Arizona University, Flagstaff, AZ USA

We established the prevalence and diversity of *Clostridium difficile* in domestic canines from Flagstaff, Arizona and compared our findings to a global database of strains known to cause human disease (www.pubmlst. org/cdifficile). Nosocomial acquisition of *C. difficile* is well documented, yet multiple studies have implicated companion pets and farm animals as possible sources of community acquired *C. difficile* infections (CDIs) in humans. To explore the potential role of pet dogs in human CDIs, we systematically collected canine fecal samples (n=197) to represent the city's densely populated neighborhoods. Additionally, nineteen fecal samples were collected from a local veterinary clinic from dogs that displayed symptoms consistent with CDIs (e.g., diarrhea). We used these samples to investigate two important questions concerning *C. difficile* carried by pet canines: (1) What is the prevalence and diversity of *C. difficile* in this companion pet population, and (2) do C. difficile isolates collected from canines genetically overlap with isolates that can cause disease in humans? We used a highly sensitive qPCR assay to initially screen all dog fecal samples for the presence of C. difficile. Culturing was then attempted on all positive samples using an anaerobic growth chamber. All cultured isolates were analyzed with two sequence-based approaches, which included multilocus sequence typing to broadly evaluate the genetic diversity of *C. difficile* in our study, followed by whole-genome sequencing to assess finer-scale patterns of diversity found within and among individual canine hosts. We detected C. difficile in 17% of the canine fecal samples, with 10% shedding toxigenic strains that are known to cause human disease. Furthermore, our sequencing analyses revealed similar genotypes and fine-scale diversity patterns in dogs and humans, and therefore, the potential for *C. difficile* transmission between them. These findings suggest that canine companion pets may play a significant but understudied role in community acquired CDIs in humans.

PIII-12 PIII-13

RIBOTYPE 017 AMONG TOXIGENIC ISOLATES ARE PREDOMINANT TOXIGENIC C. DIFFICILE IN SOUTHERN TAIWAN

Hung, Y.P.; Tsai, B.Y.; Ko, W.C.; Tsai, P.J.* Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University, Medical College, Tainan, Taiwan

Background: *Clostridium difficile* is a major cause of community-onset or nosocomial antibiotic-associated diarrhea. Ribotypes and toxin genotypes of clinical *C. difficile* isolates in Taiwan are rarely reported.

Material and Method: A prospective surveillance study from January 2011 to December 2011 was conducted at the medical wards of a district hospital in southern Taiwan. Stool samples of the patients with or without diarrhea were cultured for *C. difficile*. Only the isolates with *tcdB* were included for ribotype and toxin genotype determination, and antimicrobial susceptibility testing.

Results: Of the first toxigenic isolates from 120 patients, 68 (56.7%) of 120 isolates possessed both tcdA and tcdB. Of 52 (43.3%) with tcdB and truncated tcdA (tcdA-/tcdB+), all were ribotype 017 and none had binary toxin or tcdC deletion. Eighteen (15%) toxigenic isolates harbored binary toxins (cdtA and cdtB) and all had tcdC deletion, including Δ 39 (C184T) deletion (14 isolates), Δ 18 in-frame deletion (3), and Δ 18 (Δ 117A) deletion (1). Eleven of 14 isolates with Δ 39 (C184T) deletion belonged to the ribotype 078 family, including ribotype 127 (6 isolates), ribotype 126 (4), and ribotype 078 (1). Among 8 patients with consecutive C. difficile isolates, these isolates from 6 (75%) patients were identical, irrespective of the presence or absence of diarrhea, suggestive of persistent fecal carriage or colonization.

Conclusion: In southern Taiwan, ribotype 017 isolates with a tcdA-/tcdB+ genotype accounting for 43% (54/120) of toxigenic *C. difficile* isolates were not uncommon and of *C. difficile* isolates with binary toxin, the ribotype 078 family was predominant, accounting for 61% (11/18).

COLONIZATION OF TOXIGENIC CLOSTRIDIUM DIFFICILE AMONG ICU PATIENTS: A PROSPECTIVE STUDY

Zhang, X.; Wang, X.; Yang, J.; Zong, Z.* Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China

Background: A prospective study was performed to investigate the prevalence of colonization among ICU patients and to examine whether asymptomatic carriers were the source of subsequent *C. difficile* infection (CDI) and acquisition of toxigenic *C. difficile*.

Methods: Rectal swabs were collected from adult patients on admission to and at discharge from a 50-bed medical ICU in a major referral hospital of western China, from August to November 2014. Stools were collected from patients who developed ICU-onset diarrhea. Both swabs and stools were screened for *tcdB* (toxin B gene) by PCR. Samples positive to *tcdB* were cultured for *C. difficile* and isolates recovered were screened for *tcdB* and the binary toxin genes by PCR. Strain typing was performed using MLST and isolates belonging to the same sequence type (ST) were further typed using MLVA.

Results: During the 4-month period, rectal swabs were collected from 360 (90.9%) out of 396 patients who were admitted to the ICU. Among the 360 patients, 314 had stayed in the ICU over 3 days, of which 213 (73.6%) had a rectal swab collected within 3 days before discharge from ICU. The prevalence of toxigenic *C. difficile* colonization was 1.7% (6 cases) and 4.3% (10 cases) on admission and discharge, respectively. Only four (1.1%) out of 360 patients had CDI, corresponding to 10.7 cases per 10,000 ICU days. None of the four cases had toxigenic *C. difficile* either on admission or at discharge. Toxigenic *C. difficile* isolates were recovered from all swabs and stool samples positive to *tcdB* by PCR and belonged to 7 STs (ST2, 3, 6, 37, 54, 103, and 129). None of the isolates belonging to the same ST had identical MLVA patterns. Binary toxin genes were detected in one ST103 isolate causing colonization.

Conclusion: The prevalence of colonization with toxigenic *C. difficile* among patients on admission to ICU was low in our settings. ICU-acquired toxigenic *C. difficile* were not linked to those detected on admission. Active screening for toxigenic *C. difficile* may not be a resource-efficient measure in settings with the low prevalence of colonization.



Ihursa	ay, July 14, 2016 Clostridium difficile: Microbiol	ogy
1320	POSTER SESSION III: CLOSTRIDIUM DIFFICILE: MICROBIOLOGY	
PIII-14	Nonsteroidal Anti-Inflammatory Drugs Alter the Gut Microbiome and Increase the Severity of <i>Clostridium difficile</i> Infection in Mice <i>Trindade, B.C.; Kirk, L.; Rogers, L.M.; Zackular, J.P.; Skaar, E.P.; Schloss, P.D.; Lyras, D.; Maseda, D.; Crofford, L.J.; Aronoff, D.M.</i> *	207
PIII-15	Design of a Two-Plex Assay for Detection of <i>Clostridium difficile</i> Toxins A and B	208
PIII-16	Banz, A.;* Riou, B.; Lantz, A.; Foussadier, A. In vitro Activity of Cadazolid and Eight Comparator Antimicrobial Agents against 50 Isolates of Clostridium difficile, 379 Other Anaerobes, and 174 Aerobic Organisms Citron, D.M.;* Tyrrell, K.L.; Goldstein, E.J.C.	209
PIII-17	Toxin Profiles, PCR Ribotypes and Resistance Patterns of Clostridium difficile: A Multicenter Study in China, 2012-2013 Gao, Q.; Wu, S.; Huang, H.;* Ni, Y.; Chen, Y.; Hu, Y.; Yu, Y.	210
PIII-18	Colonization of Binary Toxin-Positive and Binary Toxin-Negative Clostridium difficile Strains in Hamsters Johnston, P.F.;* Semenyuk, E.; Siddiqui, F.; Sambol, S.P.; Driks, A.; Gerding, D.N.; Johnson, S.	211
PIII-19	Characterisation of Random <i>Clostridium difficile</i> Mutants Less Susceptible to Ridinilazole (SMT19969) in Terms of Fitness Cost Budd, P.; Kelly, M.L.; Vickers, R.J.; Winzer, K.; Minton, N.P.; Kuehne, S.A.*	212
PIII-20	In vitro Susceptibility and Genotyping of 1776 Pre-Treatment Isolates of Clostridium difficile Recovered from a Global Clinical Trial Merriam, C.V.;* Citron, D.M.; Sambol, S.P.; Wilcox, M.B.; Goldstein, E.J.C.; Dorr, M.B.	213
PIII-21	Agar Dilution versus Broth Microdilution Methods for Antimicrobial Susceptibility Testing of Clostridium difficile Nary, J.;* Citron, D.M.; Chesnel, L.; Dale, S.E.	214
PIII-22	Exploring the Diversity of Toxinotype V <i>Clostridium difficile</i> Strains through Whole Genome Sequencing <i>Norman, K.N.;* Scott, H.M.</i>	215
PIII-23	Benefits of <i>Clostridium difficile</i> Polymerase Chain Reaction Screening Followed by Toxin Confirmation <i>Robinson, P.</i> *	216
PIII-24	Analysis of Surface Proteins Obtained from <i>Clostridium Difficile</i> Brazilian Strains Cultivated with Antibiotics Ferreira, T.G; Moura, H.; Barr, J.H.; Miyajima, F.; Domingues, R.M.C.P.; Ferreira, E.O.*	217

Posters will be presented in Poster Session III Thursday, July 14 1320-1420.

PIII-25	SMT 19969: A Novel Agent for <i>Clostridium difficile</i> Infection (CDI): Synopsis of Microbiology & Phase 1 Study <i>Vickers, R.J.; Corbett, D.; Warn, P.; Citron, D.M.; Goldstein, E.J.C.; Best, E.; Wilcox, M.; Tillotson, G.S.</i> *	218
PIII-26	<i>In vivo</i> Efficacy of SMT 19969, Vancomycin, and Fidaxomicin in a Hamster Model of CDI	219
	Teague, J.; Wise, A.; Thommes, P.; Burgess, E.; Daws, G.; Payne L.J.; Vickers, R.J.; Warn, P.; Tillotson, G.S.*	
PIII-27	Comparative Exoproteome of <i>Clostridium difficile</i> Brazilian Ribotypes Treated with Subinhibitory Concentrations of Antibiotics <i>Trindade, C.N.R.;* Moura, H.; Barr, J.R.; Miyajima, F.;</i>	220

Ferreira, T.G.; Ferreira, E.O.; Domingues, R.M.C.P.

Clostridium difficile: Microbiology

Thursday, July 14, 2016

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ALTER THE GUT MICROBIOME AND INCREASE THE SEVERITY OF CLOSTRIDIUM DIFFICILE INFECTION IN MICE

Trindade, B.C.; Kirk, L.; Rogers, L.M.; Zackular, J.P.; Skaar, E.P., Schloss, P.D.; Lyras, D.; Maseda, D.; Crofford, L.J.; Aronoff, D.M.*2,3 Centro de Pesquisas René Rachou, Belo Horizonte, MG Brazil Division of Infectious Diseases, Department of Medicine, Vanderbilt University, Nashville, TN USA

³Division of Host-Pathogen Interactions, Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN USA ⁴Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI USA

⁵Department of Microbiology, Monash University, Clayton, VIC, Australia ⁶Division of Rheumatology and Immunology, Department of Medicine, Vanderbilt University, Nashville, TN USA

Background: NSAID use has been linked to dysbiosis and to *C. difficile* infection (CDI), but causal associations are lacking.

Hypothesis: We sought to test the hypothesis that NSAID exposure impacts the gut microbiota and increases CDI severity.

Methods: We used a mouse model of CDI using female C57/BL6 mice exposed to cefoperazone prior to gavage with spores of *C. difficile* (strains 630g or the NAP1 M7404). Mice were exposed to indomethacin (gavage) or ibuprofen (drinking water) either before or during infection. Some mice received a stable PGE analogue after infection. Disease severity was assessed by weight loss or death, histological inflammation, flow cytometry from the peritoneum, mesenteric nodes, and colon lamina propria, and by cytokine analysis. Microbiome of cecal contents were determined by 16S rRNA sequence analysis.

Results: Exposure to NSAIDs either during or prior to CDI increased acute colonic and peritoneal inflammation, weight loss, and mortality. Pharmacological use of a PGE analogue reduced disease severity and suppressed *C. difficile* colonization of the colon. NSAIDs significantly reduced microbial diversity independent of antibiotic use.

Conclusions: NSAID use in mice both alters the gut microbiome and increases disease severity in CDI.

Funding: this work was supported by the Vanderbilt Digestive Disease Research Center and NIH Grant DK058404

DESIGN OF A TWO-PLEX ASSAY FOR DETECTION OF CLOSTRIDIUM DIFFICILE TOXINS A AND B

Banz, A.;* Riou, B.; Lantz, A.; Foussadier, A. BioMérieux, Marcy L'Etoile, France

The purpose of this study was to evaluate the SimoaTM technology for a new assay able to quantify simultaneously each toxin, A and B, of *Clostridium (C.)* difficile in human fecal samples. The simultaneous detection of both toxins is possible thanks to the use of fluorescent paramagnetic beads and of two detectors specific to each toxin. Antibodies (Abs) with the broader pattern of detection were selected. The specificity of these Abs was evaluated. We observed that each Ab pair was specific to toxin A and B, respectively. The interference of toxin A on the accuracy of toxin B assay, and inversely was also evaluated. No impact of high concentrations of toxin A (up to 100 ng/ ml) on the accuracy of toxin B assay was observed, while concentrations of toxin B superior or equal to 10 ng/ml interfered with the accuracy of toxin A assay at low concentrations (0-10 pg/ml). Nevertheless, for toxin A concentration of 1000 pg/ml, toxin B had no impact on assay accuracy. As the positive threshold for stool samples is closed to 30 pg/ml, the accuracy of toxin A assay at this concentration and above will have to be further evaluated. For the two-plex assay, the dye-encoded beads were tested and compared to unencoded beads: similar results were obtained in term of antibody coating efficacy and limit of detection. The cross-reactivity of the Abs was then evaluated in the two-plex format. We observed that anti-toxin B Ab used as capture on beads was specific to toxin B, while anti-toxin A Ab as capture could detect some purified toxin B commercially available when anti-toxin B Ab was used as detector. As the specificity of the response is determined by the beads, this anti-toxin A Ab can be used as detector only. Finally, some low non-specific signals were observed in the two-plex assay when the response for one analyte is high, and it is due in part to an optical phenomena called spatial crosstalk.

In conclusion, even if the measuring range may be restricted in comparison to an one-plex assay, we can develop a two-plex assay on SimoaTM technology for performing fully-automated measurement of *Clostridium difficile* toxins A and B simultaneously with high sensitivity.

IN VITRO ACTIVITY OF CADAZOLID AND EIGHT COMPARATOR ANTIMICROBIAL AGENTS AGAINST 50 ISOLATES OF *CLOSTRIDIUM DIFFICILE*, 379 OTHER ANAEROBES AND 174 AEROBIC ORGANISMS

Citron, D.M.;* Tyrrell, K.L.; Goldstein, E.J.C. R.M. Alden Research Lab, Santa Monica, CA, USA

Purpose: *C. difficile* infection (CDI) continues to increase in incidence and severity with recurrence rates of 15-30% with the currently used antimicrobials. Cadazolid (CDZ) is a new quinolonyl-oxazolidinone agent in clinical development for the oral treatment of CDI. This study further evaluated the *in vitro* activity of CDZ against *C. difficile* and other gut organisms.

Methods: The isolates were mostly from stool and other intra-abdominal specimens. Anaerobes were tested using the agar dilution method [CLSI M11-A8], while aerobic organisms were tested by the broth microdilution method [CLSI M07-A10]. The comparator antimicrobials included linezolid, vancomycin, metronidazole, fidaxomicin, moxifloxacin, clindamycin, and rifaximin.

Results: CDZ MIC $_{50/90}$ values against *C. difficile* were $0.25/0.5 \,\mu g/ml$, and ranged from 0.25 - $0.5 \,\mu g/ml$ regardless of ribotype or resistance to other test agents, including four isolates with linezolid MICs of $16 \,\mu g/ml$. MICs for other *Clostridium* spp ranged from 0.125 - $1 \,\mu g/ml$, and for other Gram-positive non-spore-forming rods, they ranged from 0.06 - $1 \,\mu g/ml$. Several species of lactobacilli had higher MICs of $8 \,\mu g/ml$. *Bacteroides fragilis* and *B. thetaiotaomicron* showed a range of 2 - $4 \,\mu g/ml$, while *B. vulgatus* and *Parabacteroides* spp had a range of 2 - $32 \,\mu g/ml$. Higher MICs of 8 - $32 \,\mu g/ml$ were also found in some strains of fusobacteria and *Veillonella*. Staphylococci and enterococci had MIC $_{50/90}$ of 0.25/0.25 regardless of resistance to other antimicrobials. All Enterobacteriaceae showed MICs of $16 \,$ - $32 \,\mu g/ml$.

Conclusion: CDZ exhibited potent activity against *C. difficile*, and most other Gram-positive species tested regardless of susceptibility to other agents. Activities of CDZ against Gram-negative anaerobes was generally weaker with elevated MICs against some of the *Bacteroides*, *Veillonella*, and fusobacteria species and no or weak activity was observed against Enterobacteriaceae. As an investigational agent for treatment of CDI, the effects of CDZ upon the complex microflora present in the gut of CDI patients warrant further investigation.

PIII-17 PIII-18

TOXIN PROFILES, PCR RIBOTYPES AND RESISTANCE PATTERNS OF *CLOSTRIDIUM DIFFICILE*: A MULTICENTER STUDY IN CHINA, 2012-2013

Gao, Q.; Wu, S.; Huang, H.; Ni, Y.; Chen, Y.; Hu, Y.; Yu, Y.

- ¹ Institute of Antibiotics, Huashan Hospital, Fudan University, China
- ²Ruijin Hospital, Shanghai Jiaotong University, China
- ³ Nanfang Hospital, Southern Medical University, China
- ⁴Beijing Hospital of Ministry of Health, China
- ⁵Sir Run Run Shaw Hospital, Zhejiang University, China

To obtain a complete overview of the phenotypic and genotypic features of C. difficile isolates in China, a total of 178 nonduplicate clinical isolates of C. difficile were collected prospectively from five major teaching hospitals, representing northern, eastern, and southern China from August 2012 to July 2013. Of these 178 isolates, 16 (9.0%) were considered to be nontoxigenic due to the absence of genes encoding toxin A, toxin B, or binary toxin. Among the remaining 162 toxigenic isolates, 66 (40.7%) were toxin A-negative, toxin B-positive and 95 (58.6%) were toxin A-positive, toxin B-positive, only one isolate (0.6%) also had binary toxin. Twenty-nine different PCR ribotypes were identified, of which 017 (21%), 012 (17%), and H (17%) were the most prevalent. PCR ribotype 027 or 078 was not found. All toxigenic strains were susceptible to metronidazole, vancomycin, and piperacillin/tazobactam. Resistance to moxifloxacin, clindamycin, erythromycin, tetracycline, chloramphenicol, fusidic acid, imipenem, linezolid, or rifampicin was observed in 45.1%, 79.6%, 75.3%, 46.9%, 3.7%, 29.6%, 4.9%, 2.5%, and 12.3% of isolates, respectively. Most of ribotype 017 strains were resistant to ≥3 antimicrobial agents, e.g. resistant to clindamycin, erythromycin, and either moxifloxacin or tetracycline. Further appropriately sampled and large prospective studies are needed to enhance our understanding of the epidemiology of CDI in China.

COLONIZATION OF BINARY TOXIN-POSITIVE AND BINARY TOXIN-NEGATIVE CLOSTRIDIUM DIFFICILE STRAINS IN HAMSTERS

Johnston, P.F.;*1 Semenyuk, E.;² Siddiqui, F.;¹ Sambol, S.P.;¹ Driks, A.;² Gerding, D.N.;¹¹2 Johnson, S.¹¹² lines VA Hospital, Hines, IL USA ²Loyola University Medical Center, Chicago, IL

C. difficile infection (CDI) has drastically increased over the past 15 years, attributed in part to the emergence of a strain with increased virulence and epidemic potential, REA group BI (aka, NAP1/027). In addition to expressing known virulence factors, large clostridial toxins A and B, BI also expresses binary toxin (CDT), the role of which is still to be defined. CDT, an actin ADP-ribosyltransferase, induces epithelial cell microtubule protrusions increasing pathogen adherence in vitro; however its effect in vivo remains unknown. We hypothesize that infection with CDT+ C. difficile results in increased adherence to intestinal epithelial cells and enhanced colonization. To test our hypothesis, we sought to establish fluorescence in situ hybridization (FISH) to visualize the location of the bacterium in the CDI hamster model. Initial FISH experiments performed with C. difficilespecific 16s rRNA probes on A+B+CDT+ (strain BI17) C. difficile-infected hamster cecum and colon found no detectable bacteria, suggesting that the level of colonization at sacrifice was not sufficient for detection, given the limited time from colonization to mortality in hamsters infected with A+B+ strains. To increase time available to study colonization, we utilized nontoxigenic (A-B-) C. difficile strains that colonize, but do not cause disease. Hamsters were infected with A⁻B⁻ CDT⁺ (strain AA1p) or A⁻B⁻ CDT⁻ (strain M3) C. difficile and colonization was examined over time by culture and FISH. Both A⁻B⁻ strains colonized hamsters and detection by FISH occurred at 48 and 72 hours post-infection, when animals were highly colonized (10⁶ -10⁷ CFUs/gm stool). *C. difficile* was detected in the outer mucous layer with both strains and in close proximity to the epithelium with the CDT+ strain, AA1p. Our studies suggest FISH can be utilized to determine the effect of CDT on intestinal epithelial adherence and colonization, helping elucidate its role in CDI.

CHARACTERISATION OF RANDOM *CLOSTRIDIUM DIFFICILE* MUTANTS LESS SUSCEPTIBLE TO RIDINILAZOLE (SMT19969) IN TERMS OF FITNESS COST

Budd, P.;¹ Kelly, M.L.;¹ Vickers, R.J.;³ Winzer, K.;¹ Minton, N.P.;¹.² Kuehne, S.A.*¹.²

¹Synthetic Biology Research Centre (SBRC), Clostridia Research Group, University of Nottingham, Nottingham UK

²NIHR Nottingham Digestive Diseases Biomedical Research Unit at Nottingham University Hospitals NHS Trust and The University of Nottingham, UK

³Summit plc, Abingdon, Oxfordshire UK

Ridinilazole (SMT19969) is a novel antimicrobial under clinical development for *Clostridium difficile* infection (CDI) that is associated with minimal impact on host gut microbiota. In a recently completed clinical phase 2 trial, ridinilazole achieved statistical superiority over vancomycin on sustained clinical response. CDI is the leading cause of antibiotic associated diarrhoea, imparting a significant financial and patient welfare burden on healthcare systems. Recurrent disease is a particular concern with at least 25% of patients experiencing relapse after an initial episode of CDI. Treatment options are limited, and hence novel countermeasures are urgently needed. Here we investigated the genetic basis for reduced susceptibility to ridinilazole and additionally whether potential mutations have an effect on the fitness of *C. difficile*.

Random mutants of *C. difficile* strain CD630 were isolated on up 10x MIC of ridinilazole. These were characterised *in vitro* including MIC, growth rates, transmission electron microscopy (TEM) and motility.

In total, 9 random mutants were isolated. All strains, except mutant 4, were non motile under the conditions tested. Analysis of the TEM pictures revealed a degree of variation with respect to flagella being present. Mutant 7 and 11 showed no flagella, mutant 23 and 30 displayed less flagella than the wildtype and additionally these appeared shortened. Mutant 16 and 4 had less flagella then the positive control and finally mutant 20 seem to display a similar number of flagella to the wildtype.

We conclude that reduced susceptibility to ridinilazole seems to be associated to a fitness cost of the bacteria. Flagella have been implicated in adherence and colonisation in certain strains of *C. difficile* and their regulation has also been linked to toxin expression in a number of publications. Our data hence suggest that *C. difficile* strains with reduced susceptibility to ridinilazole may be less able to persist in a clinical setting and might potentially even be less virulent.

IN VITRO SUSCEPTIBILITY AND GENOTYPING OF 1776 PRE-TREATMENT ISOLATES OF CLOSTRIDIUM DIFFICILE RECOVERED FROM A GLOBAL CLINICAL TRIAL

Merriam, C.V.;*1 Citron, D.M.;¹ Sambol, S.P.;² Wilcox, M.B.;³ Goldstein, E.J.C.;¹ Dorr, M.B.⁴

¹R.M. Alden Research Lab, Culver City, CA USA

²VA Medical Center, Hines, IL USA

³Leeds NH Trust, Leeds UK

⁴Merck & Co. Kenilworth, NJ USA

Background: The incidence and severity of *Clostridium difficile* infection (CDI) continues to increase and rates of morbidity and mortality remain high. New treatment approaches are needed. The purpose of this project was to investigate the genotyping and *in vitro* susceptibilities of 1776 pretreatment isolates of *C. difficile* from specimens sent to our lab from a global study of actoxumab and bezlotoxumab to prevent recurrence of CDI.

Methods: Modify I and II clinical trials were conducted 2012-2015 at sites in 30 countries in North and South America, Europe and Asia, and including Israel and S. Africa. We received stool samples for culture from 317 of 382 study sites; pre-treatment isolates were tested with six antimicrobials and sent to two labs for REA and ribotyping The antimicrobials were vancomycin (VA), metronidazole (ME), clindamycin (CL), moxifloxacin (MX), fidaxomicin (FI) and rifaximin (RX), using the agar dilution method (CLSI M11-A8).

Results: 027 was by far the predominant ribotype, comprising 18.2% (324/1776) of the isolates. Most came from the US and Canada, and made up 22% and 36% of those countries' respective totals; RT 027 was also prominent in Chile, comprising 48% of its isolates, and in Poland with 76%. 37% (119/324) of RT 027 were RX-R and 89% were MX-R. RX-resistance overall was 13%, with the greatest pockets found in Poland (RT 027) and Italy (RT 018 and 356), at 57% and 49% respectively. Of the 144 isolates with VA MIC >2, 74% came from the US and Israel (RTs 027 and 137), whereas the 2.6% of strains with ME MIC>2 came mostly from Poland with RT 027 (REA BI grp), and from Chile with RT 001 (REA J grp). MX-resistance was seen in 37% of isolates overall, and did not generally overlap with RX-resistance except in RT 027. Almost all isolates (96.6%) were resistant to CL.

Conclusions: We noted decreased susceptibility (MIC 4-8) to VA and ME and increased resistance to RX and MX; certain clustering of ribotypes was apparent even within the limits of the study.

PIII-21 PIII-22

AGAR DILUTION VERSUS BROTH MICRODILUTION METHODS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *CLOSTRIDIUM DIFFICILE*

Nary, J.;*1 Citron, D.M.;² Chesnel, L.;³ Dale, S.E.¹
¹ACM Global Central Laboratory, Rochester, NY USA
²R.M. Alden Research Laboratory, Culver City, CA USA
³Merck Research Laboratories, Merck & Co., Inc., Kenilworth, NJ USA

Purpose: This study evaluated the reproducibility and agreement of broth microdilution (BMD) to the reference agar dilution (AD) method for testing 7 antibiotics against *Clostridium difficile*.

Methods: *C. difficile* isolates (n=920) were recovered from toxinpositive fecal samples obtained through two global phase 3 clinical trials conducted between 2012 and 2015. Isolates were recovered either from neat sample inoculated into cycolserine cefoxitin mannitol broth subcultured to cycloserine cefoxitin fructose agar with taurocholate (CCFA-HT, Anaerobe Systems) or alcohol shock of the sample followed by plating to CCFA-HT. Susceptibility testing was performed by BMD using a custom formulated panel containing clindamycin, rifaximin, moxifloxacin, vancomycin, metronidazole, tigecycline, and ceftriaxone in Brucella broth (Thermo-Fisher, Trek Diagnostics). Agar dilution using the CLSI-M11-A8-reference method was performed for the same antibiotics. C. difficile ATCC 700057, B. fragilis ATCC 25285, and E. lenta ATCC 43055 were used as QC strains. The isolates were tested using the same inoculum by both methods on the same day. To assure precision of the methods, approximately 10% of the isolate testing was performed independently by two laboratories using the same lots of media and antibiotics.

Results: The method to method comparison showed a >90% agreement between the two laboratories for the subset of isolates tested. For the 920 isolates, there was an overall negative bias for MICs generated from BMD as compared to AD: MICs shifted at least 1 doubling dilution lower using BMD compared to AD. The essential agreement was greater than 95% within one 2-fold dilution with AD for ceftriaxone and moxifloxacin, between 80-90% for vancomycin and metronidazole and below 40% for clindamycin, rifaximin and tigecycline.

Conclusion: Reproducibility was variable and drug dependent. The BMD method is not comparable to AD for testing the drugs used for treatment of *C. difficile* infection, vancomycin, and metronidazole. However, BMD could be used reliably to test ceftriaxone and moxifloxacin.

EXPLORING THE DIVERSITY OF TOXINOTYPE V CLOSTRIDIUM DIFFICILE STRAINS THROUGH WHOLE GENOME SEQUENCING

Norman, K.N.;* Scott, H.M. Department of Veterinary Pathobiology, Texas A&M University, College Station, TX USA

The finding of similar Clostridium difficile strains in human infection and food animals has raised concern for *C. difficile* to be a potential foodborne pathogen and source of community-acquired *C. difficile* infections (CA-CDI). Previously, we isolated *C. difficile* from a closed population of humans and swine to investigate the potential for occupational exposure. We did not find evidence of occupational exposure; however, the majority of strains isolated from both the human wastewater and swine fecal samples were classified as toxinotype V, North American Pulsed-field type 7 (NAP7). Pulsed-field gel electrophoresis is the standard typing methods for *C. difficile* in the United States, but this may not be the most suitable typing method. Next generation sequencing may provide a more discriminatory method to differentiate between strains. We conducted whole genome sequencing on 36 swine and 27 human epidemiologically related, toxinotype V strains. Library preparation was performed using Nextera XT DNA sample preparation kits to individually index strains and libraries were sequenced on the Illumina MiSeq platform. MiSeq Reporter and Geneious Pro Software were used to assemble and align sequences, determine variants, and facilitate phylogenetic analyses. Analysis of the sequencing data showed that toxinotype V strains are phylogenetically distant from toxinotype III strains and current reference genomes in NCBI are not appropriate for analyzing toxinotype V strains. Currently, we are closing several toxinotype V genomes to provide better references for more accurate variant calling and phylogenetic analyses. Whole genome sequencing will provide a more detailed view of the genotypic diversity among toxinotype V strains. The diversity and evolution of toxinotype V strains is especially important because these strains are commonly found in both food animals and human infection, and many questions still remain about potential sources for CA-CDI. Understanding the true diversity within *C. difficile* toxinotypes and North American Pulsed-field types is essential for discussions regarding standardized typing methods.

BENEFITS OF *CLOSTRIDIUM DIFFICILE* POLYMERASE CHAIN REACTION SCREENING FOLLOWED BY TOXIN CONFIRMATION

Robinson, P.*

Hoag Memorial Hospital Presbyterian, Newport Beach, CA USA

Background: Real-time polymerase chain reaction (PCR) testing quickly and reliably identifies patients who are carriers of toxigenic *Clostridium difficile* (*C. difficile*). PCR testing alone can result in over diagnosis of *Clostridium difficile* infection (CDI) leading to increased costs and overtreatment. In order to accurately identify CDI, toxin confirmation assays are required.

Objective: Evaluate a testing algorithm for CDI utilizing PCR screening followed by toxin confirmation.

Methods: All unformed or liquid stool samples collected for *C. difficile* were screened with PCR. Samples positive for Toxin B gene were frozen and sent to a reference laboratory for *C. difficile* cytotoxin assay (CTA). The same samples were also tested on site for toxin using enzyme immunoassay (EIA). CTA results were compared to EIA results.

Results: During a 7-week period, 75 PCR positive stool samples were tested for the presence of toxin by EIA and CTA. Thirty-three percent (33%) (25/75) of the PCR positive stool samples tested EIA toxin positive. One hundred percent (100%) (25/25) of the EIA toxin positive samples tested CTA positive. Forty-two percent (42%) (21/50) of the EIA toxin negative samples tested CTA positive and 58% (29/50) tested CTA negative.

Conclusions: After initial PCR screening, EIA was 100% specific but only 54% sensitive compared to CTA. Compared to CTA, the positive predictive value (PPV) of EIA was 100%, and negative predictive value (NPV) was 58%. CDI screening using PCR followed by confirmatory EIA and CTA (when EIA is negative) provides rapid and accurate CDI diagnosis. This algorithm also identifies patients who are colonized with toxigenic strains of *C. difficile* allowing targeted infection prevention and antimicrobial stewardship interventions.

ANALYSIS OF SURFACE PROTEINS OBTAINED FROM CLOSTRIDIUM DIFFICILE BRAZILIAN STRAINS CULTIVATED WITH ANTIBIOTICS

Ferreira, T.G.;¹ Moura, H.;² Barr, J.H.;² Miyajima, F.;³ Domingues, R.M.C.P.;¹ Ferreira, E.O.*¹,⁴

¹Laboratório de Biologia de Anaeróbios, IMPG, Universidade Federal do Rio de Janeiro, RJ Brasil

²Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control, Atlanta, GA USA

³Medical Research Council, University of Liverpool, Liverpool UK ⁴UFRJ-Polo Xerém, RJ Brasil

Adhesion is a critical early step in Clostridium difficile (Cdiff) colonization and involves a number of virulence factors, but the precise mechanisms by which bacteria adhere to the mucosa and initiate infection remain to be better elucidated. Thus, the aim of this study was to characterize the expression of surface-associated proteins (SP) of Brazilian Cdiff exclusive ribotypes (RT133 and RT135), comparing with worldwide circulating ribotypes 014 and 027 (NAP1), when grown under sub lethal concentrations of clindamycin and levofloxacin The antibiotics were added to the Brucella broth to a final concentration of 0.5 x MIC, and the bacteria were grown in an anaerobic cabinet at 37°C for 18h. Enriched SP fractions were obtained by using a low pH glycine lysis buffer (pH 2.2) and further analyzed using a gel-free approach combined to nLC-ESI-MS/MS mass spectrometry/ Orbitrap (MS). We have identified an average of 230 proteins for each ribotype and condition. Our results indicated that RT014 and RT135 were the most affected strains and clindamycin had the strongest effect in protein expression. We detected for all tested strains an increase up to six times for both S-layer protein precursor and flagellin when treated with antibiotics. This might improve the adherence and colonization of bacteria, and additionally promote biofilm development. Several proteins were downregulated in the presence of clindamycin, such as, putative redox-sensing transcriptional repressor; HfQ protein; activator of 2-hydroxyisocaproyl-CoA dehydratase; ABC transporter, substrate-binding lipoprotein. We confirmed that sub-lethal concentrations of antibiotics can drastically change the surface protein profile of determined Cdiff ribotypes, which can directly affect their virulence. Given the lack of an effective toxin-based vaccine for *C. difficile*, the identification of novel factors influencing the pathogenesis of this organism is essential.

Financial support: CAPES, CNPq and FAPERJ

PIII-25 PIII-26

SMT 19969: A NOVEL AGENT FOR *CLOSTRIDIUM DIFFICILE* INFECTION (CDI)- SYNOPSIS OF MICROBIOLOGY & PHASE 1 STUDY

Vickers, R.J.; Corbett, D.; Warn, P.; Citron, D.M.; Goldstein, E.J.C.; Best, E.; Wilcox, M.; Tillotson, G.S.*

¹Summit plc, Abingdon, Oxfordshire UK

²EVOTEC, Manchester UK

³R.M. Alden Research Lab, Santa Monica, CA US

⁴Leeds General Infirmary, Leeds, UK

⁵Cempra Pharmaceuticals, Chapel Hill, NC USA

Objective: CDI is a leading cause of nosocomial diarrhoea, SMT19969 is a selective antimicrobial in development for the treatment of CDI. The objective of the following is to summarise *in vitro* microbiology and Phase 1 clinical data.

Methods: MICs were determined by CLSI guidelines M11-A8 and M7-A9. Time kill assays were performed at 1-20xMIC with CFU counts to 24 hours. PAE assays were performed with either 1 or 3 hours pre-incubation with CFU counts to 24 hours post drug exposure. The primary objective of the Phase 1 was to determine safety and tolerability. 56 healthy male subjects were randomised to 8 groups. Groups A-F received single oral doses of SMT19969 or placebo escalating from 2 to 2000 mg while fasting. Group E evaluated food effect with subjects participating in 2 treatment periods (fasted and fed). Group G-H received 200mg or 500mg of SMT19969 or placebo BID for 10 days. SMT19969 was quantified in plasma and faecal samples. Safety and tolerability were assessed by adverse event (AE) monitoring, vital signs, 12-lead ECG, clinical laboratory evaluation and physical examination. Gut bacteria were cultured and quantified from Groups G and H faecal samples on days -1, 4 and 10.

Results: SMT19969 showed potent inhibition of *C. difficile* (MIC_{∞}=0.125-0.25 mg/L) against 133 clinical isolates. Against 360 Gram-positive and -negative anaerobic and aerobic bacteria, SMT19969 was more selective than fidaxomicin, vancomycin, or metronidazole comparators, with limited activity against most microorganisms including Bacteroides, Bifidobacteria, Eggerthella, Finegoldia, and Peptostreptococcus species (MIC_{oo} >512, >512, >512, 64 and 64 mg/L, respectively). SMT19969 showed bactericidal activity with >5log reduction in CFU/mL at 24 hours, and a pronounced PAE with no recovery of growth following 3 hours preincubation. In the completed Phase 1 study oral administration of SMT19969 was considered safe and well tolerated. No subjects were excluded from the analysis. AEs were mild with no dose dependent relationship and a similar rate of AEs between placebo and SMT19969 subjects. No clinically significant findings from clinical laboratory, ECGs or other assessment were observed. Plasma levels of SMT19969 were at or just above the limit of detection. There were no significant changes in gut flora bacteria except for total clostridia with mean reductions from day -1 to day 10 for Group G and H of 5.7 and 4.5 CFU/mL, respectively.

Conclusions: These data demonstrate that SMT19969 is a potent, bactericidal and selective inhibitor of *C. difficile*. Phase 1 results show that SMT19969 is safe and well tolerated with repeat administration, and has minimal impact on gut flora. These data support continued development of SMT19969 as a potential therapy for CDI.

IN VIVO EFFICACY OF SMT 19969, VANCOMYCIN, AND FIDAXOMICIN IN A HAMSTER MODEL OF CDI

Teague, J.;¹ Wise, A.;¹ Thommes, P.;¹ Burgess, E.;¹ Daws, G.;¹ Payne L.J.;¹ Vickers, R.J.;² Warn, P.;¹ Tillotson, G.S.*³ ¹Euprotec, Manchester UK ²Summit plc, Abingdon, Oxfordshire UK

³Cempra Pharmaceuticals, Chapel Hill, NC USA

Objectives: *C. difficile* is a major cause of nosocomial diarrhoea in hospitals and is increasingly a cause of CA-diarrhoea for which new treatments are required. SMT19969 (SMT), is under clinical development as a narrow-spectrum oral agent for that demonstrates potent bactericidal efficacy against *C. difficile*. We determined the efficacy & GI PK of SMT, compared to efficacy of vancomycin (vanc) & fidaxomicin (fidax) in chronic hamster models of CDAD.

Methods: *C. difficile* ribotypes (RT) 027 & 012 were used. For PK & efficacy studies hamsters were preconditioned with 30mg/kg oral clindamycin. Hamsters were infected with *C. difficile* & treatment from 20h post infection. (a) PK study SMT was 1 or 2 doses at 12.5 & 25mg/kg/dose with GI samples collected 1-24h post dose. (b) efficacy study SMT was at 12.5 & 25 mg/Kg BID, vanc at 10 mg/Kg BID, fidax at 1-25 mg/Kg BID or vehicle for 5 days (10/group). Hamsters were monitored for survival till day 28. Feces collected on days 1, 7, 12, & 19 for counts of *C. difficile*.

Results: Plasma PK of SMT were < LOD, but GI PK in the caecum & colon were >> MIC, enhanced levels of SMT measured in colon & caecum following BID dosing gave protection throughout dosing interval; stomach & small intestine PK were >> MIC for part of dosing interval. Vanc, fidax & SMT resulted in 100% survival during therapy for both RT. After end of treatment 90% -100% of vanc treated animals succumbed by day 12. For RT 027 fidax was protective with 80-100% survival, but with RT 012 hamsters succumbed from day 7 with 0-40% survival & no obvious dose response. SMT was protective against both strains with 80-100% survival. Spores counts were low or absent with vanc until shortly before death. Spores were recovered from most hamsters treated with fidax from day 7 with both RT. Spore counts were very low following SMT with most hamsters cleared.

Conclusions: SMT demonstrated predictable GI PK with no plasma exposure. SMT was superior to vanc & fidax in prolonging survival against RT 027 & superior to vanc against RT 012. These studies support the continued clinical development if SMT for the treatment of CDI.

COMPARATIVE EXOPROTEOME OF *CLOSTRIDIUM* DIFFICILE BRAZILIAN RIBOTYPES TREATED WITH SUBINHIBITORY CONCENTRATIONS OF ANTIBIOTICS

Trindade, C.N.R.;*1 Moura, H.;2 Barr, J.R.;2 Miyajima, F.;4 Ferreira, T.G.;1 Ferreira, E.O.;^{1,3} Domingues, R.M.C.P.¹

¹Laboratório de Biologia de Anaeróbios, Instituto de Microbiologia Paulo de Góes, UFRJ, Rio de Janeiro, RJ Brasil

²Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control, Atlanta, GA USA

³UFRJ- Polo Xerém, Duque de Caxias, RJ Brazil

⁴Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool UK

Clostridium difficile (Cdiff) is considered the major etiological agent of bacterial diarrhea associated with antibiotic use, thus being an important nosocomial pathogen and a significant cause of morbidity and mortality. The aim of this work is to identify the exoproteomes of two exclusive brazilian Cdiff ribotypes, RT133 and RT135, and compare them to worldwide circulating ribotypes 014 and 027 (NAP1), when exposed to sub lethal concentrations of clindamycin and levofloxacin. All strains were grown in 40 mL BHI-PRAS under anaerobic conditions. Two preparationse of culture supernatants (CS) were studied: (i) directly concentrated and trypsinized CS using on-filter digestion (Spin filter Millipore); and (ii) SDS-PAGE analysis of concentrated C followed by in-gel digestion. Digested extracts were analyzed by nLC-ESI-MS/MS mass spectrometry/Orbitrap (MS). Approximately 145 proteins were identified by MS for each strain and condition studied. Label-free quantification revealed variable amounts of a number of proteins, in special precursor S-layer, chaperonin, acyl-CoA dehydrogenase, cell surface, heat chock, rubrerythrin, and molecular chaperone DnaK. The analysis suggested that clindamycin had a stronger effect on protein expression (10-fold for some proteins in most of the tested strains). Levofloxacin also induced the expression of ferrodoxin, aldolases, Co-A transferase, glyceraldeyde phosphate proteins. The 014 ribotype was the only strain that showed an exclusive protein, a phospholipase C, in the presence of clindamycin. Phospholipase C, has previously been described in the majority of the virulent strains of *C. perfringes*. Future data analysis is underway. This work is uncovering new virulence factors that may help to clarify Cdiff pathogenicity and explain the involvement of RT133 and RT135 ins Cdiff patients in Brazil.

Financial support: FAPERJ, CNPq, CAPES

Thursda	ay, July 14, 2016 <i>C</i>	lostridium difficile: Managem	ent
1320	POSTER SESSION III: CLC MA	STRIDIUM DIFFICILE: NAGEMENT	
PIII-28	Impact of Surotomycin Treatm Bassères, E.;* Endres, B.T.; I Alam, M.J.; Chesnel, L.; Gar	Shaleduzzaman, M.; Miraftabi, F.;	223
PIII-29	Vancomycin-Resistant Enteroce C. difficile Epidemiology: The I Chesnel, L.; Devaris, D.; Citr Sears, P.		224
PIII-30	2-5° C and Ambient Temperatu	ycin (VA) Preparations Stored at res for Up to 60 Days Renaud, K.; Goldstein, E.J.C. ¹	225
PIII-31	Morphologic Changes with Ar difficile Provide Novel Insight	tibiotics Targeting Clostridium for Drug Mechanism of Action Memariani, A.; Chang, L.; Alam, M.J.;	226
PIII-32			227
PIII-33	Lactone Designed to Reduce M Methanobrevibacter smithii and t Irritable Bowel Syndrome with	hus Relieve Symptoms of Constipation; Coughlin, O.; Rezaie, A.; Wacher, V.;	228
PIII-34	A Combination of the Probiotic Germination of <i>Clostridium Dif</i> <i>Rätsep, M.; Kõljalg, S.; Sepp</i> <i>Songisepp, E.; Naaber, P.; M</i>	ficile Spores , E.; Smidt, I.; Truusalu, K.;	229
PIII-35	A Quaternary Ammonium Sol- Sensitizers Induces Susceptibil to Ambient Environmental Stre Nerandzic, M.M.;* Donskey	ution Containing Chemical ty in <i>Clostridium difficile</i> Spores essors	230
PIII-36	CDAD-Daysyms®: A New Pati Clostridium difficile-associated I	ent-Reported Outcome Tool for	231

Posters will be presented in Poster Session III Thursday, July 14 1320-1420.

Thursday, July 14, 2016 Clostridium difficile: Management

PIII-37	Molecular Basis for Neutralization of Clostridium difficile Toxins		
	by the Antitoxin Antibodies Actoxumab and Bezlotoxumab	232	
	Hernandez, L.D.; Kroh, H.K.; Beaumont, M.; Sheth, P.R.; Yang, X.; DiNunzio, E.; Rutherford, S.A.; Ohi, M.D.; Murgolo, N.J.;		
	Xiao, L.; Orth, P.; Racine, F.; Reichert, P.; Hsieh, E.; Ermakov, G.; Strickland, C.; Lacy, D.B.; Therien A.G.*		
PIII-38	Clostridium difficile: The Holistic Costs in the Era of New		
	Therapies	233	
	Tillotson. J.;* Chopra, T.; Tillotson, G.S.		
PIII-39	Ridinilazole (RDZ) for Clostridium difficile Infection (CDI):		
	Results from the Codify Phase 2 Clinical Trial	234	
	Vickers, R.J.; Wilcox, M.H.; Gerding, D.N.; Tillotson, G.S.		
PIII-40	Determining the Stability of Frozen and Lyophilized Fecal		
	Microbiota Transplant (FMT) Product Used to Treat Patients		
	with Multiple Bouts of Clostridium difficile Infection (CDI)	235	
	Valilis, E.M.;* Jiang, Z.D.; Ke, S.; DuPont, H.L.		
PIII-41	Assessment of Efficacy of Bezlotoxumab for Prevention of		
	Clostridium difficile Infection Recurrence by Diagnostic Test Method	236	
	Wilcox, M.H.;* Rahav, G.; Dubberke, E.; Gabryelski, L.; Eves, K.;		
	Tinning, R.: Guris, D.: Kartsonis, N.: Dorr, M.B.		

IMPACT OF SUROTOMYCIN TREATMENT AGAINST CLOSTRIDIUM DIFFICILE

Bassères, E.;*1 Endres, B.T.;1 Khaleduzzaman, M.;1 Miraftabi, F.;1 Alam, M.J.;1 Chesnel, L.;2 Garey, K.W.1 University of Houston College of Pharmacy, Houston, TX USA 2Merck and Co., Inc. Kenilworth, NJ USA

Surotomycin is an antimicrobial with targeted activity against *C. difficile* currently undergoing phase III clinical trials whose mechanism of action (MOA) has not been fully elucidated. This study aimed to assess the pharmacologic activity of surotomycin.

Time-kill curves were performed using the strain R20291 (BI/NAP1/027) at supra- (4x and 40x) and sub-MIC (0.25x, and 0.5x) concentrations of surotomycin. Following treatment, *C. difficile* cells were collected for CFU counts, toxin A and B production, and morphologic changes using scanning electron microscopy. Caco-2 cells were co-incubated with surotomycin-treated *C. difficile* growth media to determine the effects on host inflammatory response measured with interleukin- (IL-) 8.

Treatment at supra-MIC concentrations of surotomycin resulted in a reduction of vegetative cells over 72 hours (4 log difference, P<0.01) compared to controls without effect on spore formation. These results correlated with a 77% and 68% decrease in toxin A and B production at 48 hours, respectively (P<0.005, each), which resulted in an attenuation in inflammatory response as measured by a 55% reduction in IL-8 release compared to controls (P<0.005). Bacterial cell morphology assessed at 24 hours after 4xMIC exposure showed that the cell wall was broken apart by surotomycin treatment. From subMIC concentrations, bacterial cells presented a dose-dependent "deflated" phenotype plus a rippling effect at 0.5xMIC.

These results suggest that surotomycin has potent killing effects on *C. difficile* that reduce toxin production and attenuate the host inflammatory responses. The morphological data also confirm observations suggesting surotomycin is a membrane-active antibiotic.

VANCOMYCIN-RESISTANT ENTEROCOCCUS (VRE) COLONIZATION AND C. DIFFICILE EPIDEMIOLOGY: THE DEFLECT-1 STUDY

Chesnel, L.;*¹ Devaris, D.;¹ Citron, D.M.;² Sambol, S.P.;³ Hecht, D.;³ Sears, P.¹ Merck & Co., Inc., Kenilworth, N, USA ²R.M. Alden Research Laboratory, Santa Monica, CA USA ³ Edward Hines, Jr., Veterans Administration Hospital, Hines, IL USA

Purpose: DEFLECT-1, a randomized, placebo-controlled, double-blind study evaluated the safety and efficacy of fidaxomicin (FDX) vs. placebo (PLC) as *Clostridium difficile*-associated diarrhea (CDAD) prophylaxis in individuals undergoing hematopoietic stem cell transplantation (HSCT). *C. difficile* susceptibility, typing and VRE colonization were compared across both arms.

Methods: Screening and End of Treatment/Early Termination (EOT/ET) samples were tested for colonization by VRE. At any Unscheduled Visit (USV) where CDAD was suspected, a stool sample was collected and assayed for the presence of toxigenic *C. difficile*. Positive samples were shipped to the central microbiology laboratory for isolation, culture, restriction endonuclease analysis (REA), antibiotic susceptibility testing.

Results: *C. difficile* was isolated at baseline from 44 (7.3%) subjects in the mITT population, 16 subjects in the FDX arm and 28 subjects in the PLC arm. The most common REA group was non-specific REA (16 subjects), followed by Y group (10 subjects). Rifaximin had the lowest $\text{MIC}_{50'}$ $\text{MIC}_{90'}$ and geometric mean, followed by FDX. The types and susceptibilities of isolates were similar in both treatment arms. At Screening, 13.2% and 12.3% of subjects in the FDX and PLC arms were colonized with VRE. At follow-up (EOT/ET or USV), VRE colonization was significantly lower in the FDX arm (15.6%) vs. the PLC arm (26.4%; p=0.0058). The proportion of subjects negative at Screening, but positive at EOT/ET or USV was 2-fold higher in the PLC arm (17.7%), as compared to the FDX arm (8.4%; p=0.0090).

Conclusions: All *C. difficile* strains were susceptible to FDX in both treatment groups with MIC distribution within the wild type range. No changes in susceptibility were observed upon FDX exposure. The incidence of VRE colonization was significantly lower in the FDX arm (15.6%) than in the PLC arm (26.4%) at follow-up (p=0.0058). The proportion of subjects who developed VRE post-transplant was 2-fold higher in the PLC arm (17.7%) versus the FDX arm (8.4%; p=0.0090).

STABILITY OF THREE ORAL VANCOMYCIN (VA) PREPARATIONS STORED AT 2-5°C AND AMBIENT TEMPERATURES FOR UP TO 60 DAYS

Citron, D.M.;*1 Tyrrell, K.L.;¹ Renaud K.;² Goldstein, E.J.C.¹ ¹R.M. Alden Research Lab, Culver City, CA USA ²CutisPharma, Inc., Wilmington, MA USA

Background: Oral VA is used to treat *Clostridium difficile* infection. Several different preparations are available including reconstituted IV solutions, vancocin capsules and grape flavored FIRST Vancomycin (FIRST), (CutisPharma). The shelf life for IV after reconstitution varies between 7-14 days under refrigeration, and a standard 30 days for FIRST.

Methods: The impact of storage on the potency of VA was determined in 3 different preparations by measuring MICs for 25-50 strains of *C. difficile* and *Staphylococcus aureus*, at T₀, after 14, 30, and 60 days, stored at ambient (RT) and 2-5°C. The agar dilution (AD) method was used for anaerobes, and broth microdilution (BMD) for staphylococci. The controls were VA reference standard and FIRST diluent without VA, the latter developed to inhibit the growth of mold and yeast. All serial two-fold dilutions were prepared on the same day and added to molten agar to prepare plates for AD tests. The MIC was defined as the lowest dilution that showed no growth or a major decrease in growth. For *S. aureus*, BMD plates were prepared and frozen at -70°C until used. After inoculation, they were incubated at 35°C for 18-20h.

Results: The triplicate MICs (µg/ml) were averaged and the geometric mean calculated for all strains, preparations and time periods. The geometric mean MICs for each preparation and organism at T_0 14, 30, and 60d at RT and 2-5C were less than one dilution different for all of the values and thus considered insignificant. The FIRST diluent passed as a negative control. When stored at RT for 60 days, FIRST and IV prep showed no growth; however, the IV and capsule preps had a ground-glass, cloudy appearance, and the capsule prep grew a mold at a concentration of 4 X 10^5 CFU/ml. All preparation's MICs were comparable to the VA control, including FIRST that was stored for 21 months.

Conclusion: VA preparations showed stability over a period of 60 days regardless of storage conditions. However, the tablet preparation showed mold after 60d at RT, but unlike FIRST, which retained a clear appearance, the IV and capsule preps showed evidence of crystallization.

PIII-31 PIII-32

MORPHOLOGIC CHANGES WITH ANTIBIOTICS TARGETING CLOSTRIDIUM DIFFICILE PROVIDE NOVEL INSIGHT FOR DRUG MECHANISM OF ACTION

Endres, B.T.;*1 Bassères, E.;1 Memariani, A.;3 Chang, L.;2 Alam, M.J.;1 Kakadiaris, I.A.;3 Chesnel, L.,4 Garey, K.W.1

¹Department of Pharmacy Practice and Translational Research

²Department of Electrical and Computer Engineering

³Department of Computer Science, University of Houston, Houston, TX USA

⁴Merck and Co., Inc. Kenilworth, NJ USA

Assessment of antibiotic mechanism of action (MOA) with new drug development directed towards anaerobic bacteria is difficult and technically demanding. To gain insight into possible MOA, morphologic changes associated with antibiotic exposure can be visualized using scanning electron microscopy (SEM). SEM along with traditional kill curves may improve our insight into drug MOA and advance the drug development process. To test this hypothesis, kill curves and SEM studies were conducted using drugs with known but different MOA (vancomycin, metronidazole, and fidaxomicin). C. difficile cells (R20291) were grown with or without the presence of antibiotic for up to 48 hours. Throughout the 48 hour interval, cells were collected at multiple time points to determine antibiotic efficacy and for imaging on the SEM. Consistent with previous reports, vancomycin, metronidazole, and fidaxomicin had significant bactericidal activity following 24h of treatment (>4 log₁₀ difference for all antibiotics; *P*<0.05 compared to control). Using SEM imaging and a semi-automatic pipeline for analyzing the images, vancomycin was shown to significantly affect the cell wall ($+\Delta 116\%$ in cell wall deformation; P<0.05) compared to other antibiotic treatments and control cells. In contrast, fidaxomicin and metronidazole had significant effects on cell length (>50% reduction in cell length for each antibiotic; P<0.05) compared to controls and vancomycintreated cells. While the phenotypic response to drug treatment has not been documented previously in this manner, they are consistent with the drug's MOA demonstrating the versatility and reliability of the imaging and measurements and the application of this technique for other experimental compounds.

RECURRENT CLOSTRIDIUM DIFFICILE INFECTION AND COLONIZATION IN THE 12 MONTHS FOLLOWING ADMINISTRATION OF ACTOXUMAB AND BEZLOTOXUMAB

Goldstein, E.J.C.;*¹ Citron, D.M.;¹ Gerding, D.N.;² Wilcox, M.H.;³ Tipping, R.;⁴ Dorr, M.B.;⁴ Gabryelski, L.;⁴ Eves, K.;⁴ Kartsonis, N.;⁴ Pedley, A.⁴¹R.M. Alden Research Laboratory, Santa Monica, CA USA²Hines VA Hospital, Hines, IL USA³University of Leeds, Leeds UK⁴Merck & Co., Inc., Kenilworth, NJ USA

Purpose: MODIFY II was a global, randomized, double-blind, placebocontrolled trial of actoxumab (ACT) and bezlotoxumab (BZO), monoclonal antibodies which neutralize *C. difficile* toxins A and B, in adults receiving standard of care antibiotics for primary or recurrent clostridium difficile infection (rCDI). The trial showed that ACT+BZO (14.9%) or BZO alone (15.7%) is superior to placebo (PBO, 25.7%) in prevention of rCDI through 12 weeks (both p \leq 0.0003). We summarize the results from the subset of \sim 300 subjects enrolled in an extension study and followed for up to 12 months.

Methods: Loose stool counts and sampling for toxigenic *C. difficile* testing were carried out for subjects with a new diarrheal episode. Also, stool samples were collected at Month 6, 9, and 12 visits to assess for *C. difficile* colonization using culture with toxin testing.

Results: Only 3 subjects experienced rCDI during the extension phase of the study (ACT+BZO: n=2; PBO: n=1) among those who resolved their initial episode and had no rCDI during the 12 weeks of the main study. The cumulative incidence of rCDI in all enrolled subjects through week 12 was 14.9% in the ACT+BZO group, 15.7% in the BZO group and 25.7% in the PBO group; and was 21.4% in the ACT+BZO group, 16.2% in the BZO group and 42.7% in the PBO group through month 12 in subjects enrolled in the extension phase. *C. difficile* colonization rates among subjects who provided a stool sample were similar across treatment groups at each follow-up time point with ranges of 16.3% and 24.7% in the ACT+BZO and BZO groups and 18.8% and 32.8% in the PBO group.

Conclusion: In this extension cohort, rCDI rates increased marginally when follow up time was extended from 12 weeks to 12 months. *C. difficile* colonization rates were similar in antibody vs PBO recipients. These findings suggest that the efficacy of ACT+BZO and BZO seen in the main study is due to prevention of rCDI rather than a delay in onset of a rCDI episode.

SYN-010, A NOVEL, MODIFIED-RELEASE FORMULATION OF LOVASTATIN LACTONE DESIGNED TO REDUCE METHANE PRODUCTION BY *METHANOBREVIBACTER SMITHII* AND THUS RELIEVE SYMPTOMS OF IRRITABLE BOWEL SYNDROME WITH CONSTIPATION

Kokai-Kun, J.F.;*¹ McFall, H.;¹ Coughlin, O.;¹ Rezaie, A.;² Wacher, V.;¹ Pimentel, M.;² Gottlieb, K.;¹ Sliman, J.¹ Synthetic Biologics, Inc., Rockville, MD USA ²Gastroenterology, Cedars-Sinai Medical Center, Los Angeles, CA USA

Methane (CH₄) production in humans is due to methanogenic archaea in the intestine, predominantly Methanobrevibacter smithii. CH, had been perceived to produce no ill effects in humans aside from gaseous distention, but recent research suggests that a significant percentage of patients with irritable bowel syndrome with constipation (IBSC) excrete CH₄, and elevated CH₄ production may correlate with constipation and other related symptoms of IBSC. SYN-010 is a novel, modified-release formulation of lovastatin lactone which inhibits CH₄ production by M. smithii. It appears that the lactone form of lovastatin may compete with natural ligands of a key F420-dependent methanogenesis enzyme. In a recently completed clinical trial, subjects with IBS-C and a breath CH₄ > 10 ppm at screening were randomly assigned to receive placebo or SYN-010 at doses of 21 mg or 42 mg orally once daily for 28 days. Breath CH, and symptoms of IBS-C were monitored. The trial enrolled 62 subjects in three groups. The mean reduction from baseline in AUC of breath CH, production at Day 7 was greater in the SYN010 42 mg group compared with placebo but not statistically significant (SS). At Day 7, in paired tests where each subject served as their own control, SS reductions in breath methane levels were seen in the 42 mg dose arm (p = 0.02) but not the 21 mg-dose arm (p=0.64). At Day 28 however, the within group reductions were statistically significant for both SYN-010 groups. The percentage of weekly abdominal pain intensity and stool frequency responses also demonstrated improvement in the SYN-010 dosed patients. SYN-010 was well tolerated, and no serious adverse events were observed. These encouraging results from this small proof of concept study are consistent with a perceived role for CH₄ production in IBS-C. Additional clinical trials with SYN-010 for IBS-C are planned.

228

A COMBINATION OF THE PROBIOTIC AND PREBIOTIC PREVENTS THE GERMINATION OF *CLOSTRIDIUM DIFFICILE* SPORES

Rätsep, M.;^{1,2} Kõljalg, S.;¹ Sepp, E.;¹ Smidt, I.;¹ Truusalu, K.;¹ Songisepp, E.;² Naaber, P.;¹ Mikelsaar, M.*^{1,2}

¹Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

²Bio-Competence Centre of Healthy Dairy Products, Estonia

C. difficile (Cd) infection is one of the most prevalent health-care associated infections in hospitals and nursing homes. Absence of intestinal lactobacilli has been associated with Cd colonization in hospitalized patients (Naaber et al. 1997; 1998). Different approaches are used for prevention of Cd infections: (a) stewardship of antibiotic treatment, (b) application of antagonistic beneficial bacteria (probiotics) and prebiotics, (c) interference measures against germination of *C. difficile* spores. Our aim was to test (a) the susceptibility of Cd strains of different origin and the insensitivity of intestinal lactobacilli to various antimicrobials; (b) the susceptibility of Cd strains to antagonistic effects of the probiotic L. plantarum Inducia DSM 21379 (Inducia), prebiotic xylitol (Xyl), and their combination as a symbiotic (Syn); (c) the suppression of germination of Cd spores in vitro and *in vivo* animal model of *Cd* infection with Syn treatment. All reference and tested clinical isolates of Cd were susceptible to vancomycin and metronidazole. Conversely, Lactobacillus spp. strains were not susceptible to metronidazole and some lactobacilli species, incl. inducia to vancomycin. We found that metronidazole is the most suitable treatment of *Cd* infection meanwhile saving lactobacilli followed by vancomycin. Intact cells of Inducia, natural and neutralized cell free supernatant inhibited in vitro the growth of Cd reference strains and Estonian and Norwegian clinical isolates of Cd after co-cultivation. This effect against Cd sustained in liquid media under ampicillin (0.75 μ l/ml) and Xyl (0.1%, 1%, 2.5%, 5%) application. Further, pre-incubation of Inducia in the media containing 5% Xyl fully stopped germination of spores of Cd VPI strain10463 (ATCC 43255) after 48 h. In hamster *Cd* infection model, the administration of Inducia for 5d before ampicillin treatment and inoculation with 10-30 spores of Cd was followed under the treatment with Syn for 5 days. In Xyl administration the survival of hamsters reached 56% vs. 13% in Cd VPI control and in Syn to 78% (p=0.003). Thus, the combination of xylitol with *L. plantarum* Inducia suppresses the germination of spores into vegetative cells of *C. difficile* able for toxin production (US15/132.286, filed 19.04.2016). The possibility to apply antimicrobial treatment of Cd infection saving intestinal lactobacilli and pretreatment with xylitol and the antagonistic probiotic has to be tested in clinical trials.

A QUATERNARY AMMONIUM SOLUTION CONTAINING CHEMICAL SENSITIZERS INDUCES SUSCEPTIBILTY IN CLOSTRIDIUM DIFFICILE SPORES TO AMBIENT ENVIRONMENTAL STRESSORS

Nerandzic, M.M.;* Donskey, C.J. Department of Veterans Affairs, Cleveland, OH USA

Purpose: Prevention of *Clostridium difficile* transmission is challenging because dormant spores have several protective barriers that shield the vulnerable spore core from commonly used disinfectants. We hypothesized that exposing dormant spores to chemical sensitizers applied in a quaternary ammonium matrix would reduce the burden of spores on surfaces by increasing susceptibility to ambient environmental stressors such as oxygen, desiccation, benign disinfecting room lighting, and low pH.

Methods and Results: Hospital surfaces (bedrail, call button, bedside table, and mattress) were artificially contaminated with 6 logs of dormant *C. difficile* spores. After a single exposure to a quaternary ammonium solution containing sensitizers and subsequent ambient desiccation, recovery of treated spores was reduced by 1.5 logs within 2 hours, >2 logs after 24 hours, and >3 logs after 1 week, compared to spores exposed to quaternary ammonium or water alone (*P* <0.01 for each comparison). Spores that were exposed to chemical sensitizers were susceptible to hydrochloric acid at pH 2.0 after 10 minutes of exposure, whereas dormant spores remained resistant, suggesting that sensitized spores remaining on surfaces that are ingested may be killed by stomach acid. Additionally, chemically sensitized spores were reduced by a novel ceiling fixture that emits blue light at 405nm after 24 hours of exposure, while optically dense dormant spores remained unaffected.

Conclusions: Taken together, these data demonstrate that exposing spores to sensitizers in a quaternary ammonium matrix represents a novel approach for reducing environmental contamination by altering the spores, leaving them susceptible to ambient room conditions and enhancing killing by other benign agents.

230

CDAD-DAYSYMS®: A NEW PATIENT-REPORTED OUTCOME TOOL FOR *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA (CDAD)

Kleinman, L.;¹ Talbot, G.H.;² Schüler, R.;³ Broderick, K.;⁴ Revicki, D.;⁵ Nord, C.E.*⁶

¹Evidera, Seattle, WA USA

²Talbot Advisors LLC, Anna Maria, FL USA

³Actelion Pharmaceuticals Ltd, Allschwil, Switzerland

⁴Cubist Pharmaceuticals, Lexington, MA USA

⁵Evidera, Bethesda, MD USA

⁶Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Patient-reported outcome (PRO) measures provide information on how patients function and feel about their health. Despite the importance of symptom assessment in CDAD, there is no validated PRO for CDAD. A qualitative research study was conducted to develop a CDAD PRO according to US FDA PRO guidelines. Content development comprised 2 study phases, with input from an advisory group of 5 CDAD experts in Europe and North America. Phase I elicited patients' experiences of CDAD symptoms in open-ended discussions during telephone interviews. Supplementary interviews obtained nurses' observations. A draft PRO was developed following demonstration of concept saturation. Phase II involved 2 rounds of patient interviews, with revision of the draft PRO after each round. All patients were ≥18 years old, with confirmed CDAD. IRB approval and participant informed consent were obtained. Phase I interviews included 18 patients and 6 nurses in the United States; 16 additional patients were interviewed in Phase II. Patients were representative of the general CDAD population, and diverse in age, gender, and disease severity. Concept saturation was reached in Phase I for spontaneously reported CDAD symptoms. Items were organized in a draft conceptual framework with 5 hypothesized domains: diarrhea, abdominal discomfort, tiredness, lightheadedness, and other symptoms. Phase II demonstrated initial content validity of the 13-item draft daily diary (CDAD-DaySyms®). Participants reported the questions were clear, relevant, and comprehensive; were able to use the instructions to complete the diary correctly; and considered the 24hour recall period appropriate. The CDAD-DaySyms® captures symptoms relevant to CDAD patients, demonstrating initial content validity. To allow its use in clinical practice and CDAD clinical studies, final content and psychometric validity are being evaluated in 2 ongoing international clinical trials.

PIII-37

MOLECULAR BASIS FOR NEUTRALIZATION OF CLOSTRIDIUM DIFFICILE TOXINS BY THE ANTITOXIN ANTIBODIES ACTOXUMAB AND BEZLOTOXUMAB

Hernandez, L.D.;¹ Kroh, H.K.;² Beaumont, M.;³ Sheth, P.R.;¹ Yang, X.;¹ DiNunzio, E.;¹ Rutherford, S.A.;² Ohi, M.D.;² Murgolo, N.J.;¹ Xiao, L.;¹ Orth, P.;¹ Racine, F.;¹ Reichert, P.;¹ Hsieh, E.;³ Ermakov, G.;³ Strickland, C.;¹ Lacy, D.B.;² Therien A.G.*¹ ¹Merck & Co, Inc, Kenilworth, NJ USA ²Vanderbilt University, Nashville, TN USA ³Merck & Co, Inc, Palo Alto, CA USA

Clostridium difficile is an anaerobic bacterium that causes infections of the colon in susceptible patients. The organism expresses two exotoxins, TcdA and TcdB, which target the epithelial cells of the gut and cause the damage and inflammation which underlie the symptoms of C. difficile infection (CDI). The toxins are thought to bind to specific cell-surface receptors in part via their C-terminal combined repetitive oligopeptide (CROP) domains. The neutralizing antibodies actoxumab and bezlotoxumab, specific for TcdA and TcdB respectively, are protective in animal models of CDI. Furthermore, bezlotoxumab alone reduced the rate of recurrent CDI in phase 3 clinical trials. In this study, we characterize the binding of actoxumab and bezlotoxumab to their respective toxins and identify their mechanisms of neutralization. The epitopes of each antibody were identified using a combination of Western blotting, surface plasmon resonance, hydrogen-deuterium exchange—mass spectrometry, X-ray crystallography, and negative stain electron microscopy. The nature of the immune complexes formed between the antibodies and toxins was investigated using size exclusion chromatography coupled with multi-angle laser light scattering. Our data show that actoxumab and bezlotoxumab bind to two homologous but distinct epitopes within the CROP domains of TcdA and TcdB, respectively. Binding of actoxumab to TcdA causes formation of large immune complexes, whose sizes increase at higher antibody:toxin ratios, whereas binding of bezlotoxumab to TcdB does not. Despite these differences, the mechanisms of toxin neutralization are similar; each antibody prevents binding of their respective toxin to host cells, thereby blocking the intoxication cascade at its first step. These mechanisms of neutralization are presumed to underlie the efficacy of the antibodies in preclinical models of CDI and of bezlotoxumab alone in prevention of recurrent CDI in patients.

CLOSTRIDIUM DIFFICILE: THE HOLISTIC COSTS IN THE ERA OF NEW THERAPIES

Tillotson. J.;*1 Chopra, T.;² Tillotson, G.S.¹ ¹GST Micro, Chapel Hill, NC USA ²Detroit Medical Center, Detroit, MI USA

Background: *Clostridium difficile* infections (CDI) occur in 453,000 patients annually in the USA. Of these 65% are healthcare associated with 24% occurring during hospitalization. Recurrence occurs in 83,000 patients. There have been multiple evaluations of the costs of these infections, but the overall or holistic impact. The potential impact of new therapies at a US national level is reported.

Methods: For the period 2010-2015 we reviewed PubMed, SCOPUS, Science Direct, Google Scholar and EMBASE databases using following keywords, *Clostridium difficile*, economics, costs, cost-effectiveness, impact models, and healthcare systems.

Results: 64 references were identified. These were reviewed by two authors, (JT & GT) and confirmed by TC and analyzed for attributable costs including drug costs (where specified), office visits, hospital admissions, length of stay, CDI related interventions such as surgery, and re-admissions within 30 days. These studies conducted in the following countries, USA, UK, France, Germany, Italy, and Spain. The estimated drug and medical costs were collected locally. Using consensus estimates of recurrence rates and assuming sustained clinical response (SCR) rates for current (metronidazole, vancomycin, and fidaxomicin) and projected costs of potential new compounds. We were unable to stratify CDI by severity which could impact the overall analysis but assuming increases of SCR we showed differences in overall costs in management of CDI by therapy. Heimann *et al* (1) illustrated the impact of CDI recurrence on costs in terms of overall length of stay, but Gabriel (2) highlighted the impact of re-admission on costs.

Conclusions: Modern healthcare costs are complex and disease which are known to be recurrent have unseen expense. It is worth evaluating the drug costs of a new agent in light of the system-wide or holistic expense of CDI.

- (1) Heimann et al Infection 2015, DOI 10.1007/s15010-015-0810-x
- (2) Gabriel J Hosp Inf 2014, 88; 12-21

RIDINILAZOLE (RDZ) FOR *CLOSTRIDIUM DIFFICILE* INFECTION (CDI): RESULTS FROM THE CODIFY PHASE 2 CLINICAL TRIAL

Vickers, R.J.;¹ Wilcox, M.H.;² Gerding, D.N.;³ Tillotson, G.S.*⁴ ¹Summit plc, Abindon UK ²Leeds General Infirmary, Leeds UK ³VA Hines, Chicago IL USA ⁴GST Micro, Downingtown, PA USA

Background: 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 additional safety and efficacy data from the CoDIFy proof-of-concept Phase 2 clinical trial.

Methods: 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.

Results: 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). There were no clinically important differences in overall adverse events (AEs) or serious adverse events (SAEs) between groups. A total of 41 vs 40 AEs and 8 vs 9 SAEs were recorded for RDZ and VAN, respectively. RDZ was associated with reduced gastrointestinal AEs (40% vs 56%). Two deaths on the study were both in the VAN arm. Administration of RDZ resulted in day 5 faecal concentrations significantly above MIC (mean 1298 ug/g; SD 1302) and low systemic exposure with mean day 5 plasma concentrations of 0.16 ng/mL (SD 0.26).

Conclusion: 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.

DETERMINING THE STABILITY OF FROZEN AND LYOPHILIZED FECAL MICROBIOTA TRANSPLANT (FMT) PRODUCT USED TO TREAT PATIENTS WITH MULTIPLE BOUTS OF *CLOSTRIDIUM DIFFICILE* INFECTION (CDI)

Valilis, E.M.;*¹ Jiang, Z.D.;² Ke, S.;² DuPont, H.L.² ¹UTHealth Medical School, Houston, TX USA ²UTHealth School of Public Health, Houston, TX USA

Objectives: Fecal microbiota transplant (FMT) is the most effective therapy in treating patients suffering from recurrent *Clostridium difficile* infection (CDI). This study determined the stability of FMT bacterial product stored past three months. Collecting and freezing samples is an expensive task considering the high cost of screening donors. Determining if frozen and/or lyophilized samples are stable after 3 months of storage would be a significant savings and would allow treatment of greater numbers of patients.

Methods: To determine the viability of 50 frozen samples and 16 lyophilized microbiota products from different time points, quantitative polymerase chain reaction (qPCR) was used on extracted fecal DNA to enumerate the bacterial genera present in the human gut: *Bacteroidetes, Clostridium, Escherichia coli, Bifidobacterium, Lactobacillus*. Mean number of 16S rRNA sequences per μg of sample DNA was compared between each time point using non-parametric Mann-Whitney test.

Results: The frozen samples were significantly different from each other across all time points and bacterial genera (p<0.001). The same was true for lyophilized samples (p<0.001). Among frozen and lyophilized samples, levels of *Bifidobacterium* increased over time. The microbial diversity of frozen and lyophilized were statistically different among the genera *Bacteriodetes* (p=0.0060), *Bifidobacterium* (p<0.0001), *E. coli* (p=0.0029), and *Lactobacillus* (p=0.0003) with lower counts of these genera in lyophilized samples. *Clostridium* species had statistically similar levels between frozen and lyophilized samples (p=0.6531).

Conclusions: Analysis of qPCR results show that microbial content was marginally different across all time points among frozen and lyophilized samples. These differences may be attributed to the large diversity of flora across healthy donors. Frozen samples maintained greater microbial stability than lyophilized samples. The *in vivo* study using a mouse model is underway to determine the efficacy of these products on recurrent CDI in mice.

ASSESSMENT OF EFFICACY OF BEZLOTOXUMAB FOR PREVENTION OF CLOSTRIDIUM DIFFICILE INFECTION RECURRENCE BY DIAGNOSTIC TEST METHOD

Wilcox, M.H.;*¹ Rahav, G.;² Dubberke, E.;³ Gabryelski, L.;⁴ Eves, K.;⁴ Tipping, R.;⁴ Guris, D.;⁴ Kartsonis, N.;⁴ Dorr, M.B.⁴

¹University of Leeds, Leeds UK

²Sheba Medical Center, Ramat Gan, Israel

³Washington University School of Medicine, St Louis, MO USA

⁴Merck & Co., Inc., Kenilworth, NJ USA

Purpose: MODIFY I and MODIFY II were global, randomized, double-blind, placebo (PBO)-controlled trials of bezlotoxumab (BZO), a monoclonal antibody which neutralizes *C. difficile* toxin B, in adults receiving standard of care antibiotic (SOC) for primary or recurrent *C. difficile* infection (rCDI). The trials showed that BZO is superior to PBO in prevention of rCDI through 12 weeks (p=0.0003 in each trial). Clinical cure (CC) rates were similar in BZO vs PBO groups. As methods may vary in their predictive value for true CDI, we examined efficacy outcomes by baseline CDI diagnostic testing method, as a post hoc analysis.

Methods & Results: The trials required a positive stool test for toxigenic *C. difficile* before study entry. The permitted methods included cell cytotoxicity assay (CCA), culture with toxin detection or strain typing, and commercial toxin and PCR assay kits. All kits had a manufacturer stated specificity of ≥94% and detected (at least) toxin B or the gene for toxin B. Data from MODIFY I and MODIFY II were pooled. A toxin EIA kit was the most common method used at baseline (48.1%) followed by PCR (44.6%), culture (5.8%), and CCA (1.4%). Within each treatment group, CC rates were slightly lower among subjects whose diagnosis was based on PCR compared with toxin EIA (BZO: 79.0% vs 82.3%; PBO 77.4% vs 83.1%). BZO subjects had a higher rate of rCDI when diagnosed by PCR compared with toxin EIA (19.6% vs 14.5%); rCDI rates were similar in PBO subjects who were diagnosed with EIA or PCR (26.1% vs 27.3%). Thus, the observed difference in rCDI rates in the BZO vs PBO groups was larger in patients diagnosed with EIA (-12.8%) compared with those diagnosed by PCR (-6.5%).

Conclusions: The reduction in rCDI rates with BZO was at a magnitude that is clinically meaningful, regardless of the diagnosis test method. The magnitude of reduction of rCDI achieved by BZO is higher if toxin detection rather than PCR is used to diagnose CDI.

Poster Index



	CLINICAL ABSTRACTS	
PI-1	Antibiotic Susceptibility of Anaerobes Isolated from Infected Wound Sites and Blood of Orthopedic Patients with Septicemia Ayepola, O.O.;* Egwari, L.O.; Nwokoye, N.N.; Olubi, O.O.;	89
	Faparusi, J.; Babalola, F.	
PI-2	Septicaemia Following Orthorpedic Wound Infections	90
	Ayepola, O.O.;* Egwari, L.O.; Nwokoye, N.N.; Olubi, O.O.; Faparusi, F.	
PI-3	Prevalence and Antimicrobial Resistance of Anaerobic Bacteria in Infected Breasts of Turkish Women with Lactational Mastitis, Periductal and Granulomatous Mastitis *Bahar-Tokman, H.;* Taner, Z.; Velidedeoglu, M.; Goksoy, E.;	91
	Celik, V.; Demirci, M.; Dal, F.; Kucuk, Y.; Mete, B.; Yemisen, M.; Kocazeybek, B.; Gunaydın, M.; Kiraz, N.	
PI-4	Genomic Comparison of Multiple Campylobacter Rectus Strains	92
	Blackburn, D.;* Peeler-Fletcher, S.A.; Kinder, M.N.; Konganti, K.; Harrell, E.A.; Threadgill, D.S.	
PI-5	Effect of Previous Beta-lactam Therapy on Resistance to Beta- lactam Antibiotics in Gram Negative Bacteria Isolated from Cases of Chronic Suppurative Otitis Media	93
	Egwari, L.O.;* Nwokoye, N.N.; Olubi, O.O.	
PI-6	Colorectal Cancer and Fusobacterium Nucleatum Infection	94
	Flynn, K.J.;* Schloss, P.D.	
PI-7	Antibiotic Susceptibility Profiles of Recent European Anaerobes: Test 2014-2015	95
	Hackel, M.;* Bailey-Person, M.; Sahm, D.; Leister-Tebbe, H.	
PI-8	Evaluation of Two Different Systems Installed on VITEK MS System for Identification of Clinically Isolated Anaerobic Bacteria	96
	Hayashi, M.;* Tanaka, K.	
PI-9	Investigation of the Antibiotic Resistance Gene Content of Intestinal Normal Flora <i>Bacteroides</i> Strains Using a Novel Chromogenic Agar for the Isolation	97
	Sóki, J.; Jeverica, S.; Tierney, D.; Perry, J.D.; Nagy, E.;* Urbán, E.	
PI-10	Detection and Preliminary Characterization of a Novel Mobilizable Transposon, MTnBf8, Harboring a Chromosomal nimB Gene	98
	Sóki, J.; Nagy,E.;* Urbán, E.	, ,
PI-11	Epidemiology of Antibiotc Resistance of Clinically Relevant Bacteroides fragilis Group Isolates in Hungary	99
	Sarvari, P.K.; Nagy, E.;* Miszti, C.; Sóki, J.; Urbán, E	
PI-12	Antimicrobial Susceptibility of <i>Propionibacterium acnes</i> Isolated from Ecuadorian Patients Who Were Under Anti-Microbial Therapy	100
	Solís, M.B.;* Velasco, N.; Gonzalez, C.; Dressendorfer, L.M.; Zurita, J.	



PI-13

PI-14

PI-15

PI-16

PI-17

PI-18

PI-19

PI-20

PI-21

PI-22

PI-23

PI-24

Poster Index

112

113

\sim	
Antimicrobial Susceptibilities for 331 Strains of <i>Prevotella</i> Species Isolated in Japan <i>Yamagishi, Y.;* Suematsu, H.; Nishiyama, N.; Koizumi, Y.; Mikamo, H.</i>	101
Antimicrobial Susceptibilities for 67 Strains of Peptostreptoccus anaerobius Isolated in Japan Mikamo, H.; Yamagishi, Y.;* Suematsu, H.; Nishiyama, N.; Koizumi, Y.	102
The Role of Anaerobic Bacteria in Polymicrobial Necrotizing Wound Infections Zhao-Fleming, H.H.,* Rumbaugh, K.P.	103
The Role of Anaerobic Bacteria in Polymicrobial Infections <i>Zhao-Fleming, H.H.;* Rumbaugh, K.P.</i>	104
NON-DIFFICILE CLOSTRIDIA	
Comparison of <i>Clostridium perfringens</i> Toxin Genes in Isolates from Broiler Chickens Deemed Healthy, and Those with Clinical and Subclinical Enteritis *Brown, J.C.S.;* Dixon, R.A.	106
Mepacrine Reduces Caco-2 Cell Death Caused by <i>Clostridium</i> perfringens Enterotoxin (CPE) Freedman, J.C.;* McClane, B.A.	107
Chronic Osteomyelitis Caused by Clostridium hydrogeniformans Hirai, J.;* Yamagishi, Y.; Sakanashi, D.; Suematsu, H.; Kinjo, T.; Fujita, J.; Mikamo, H.	108
Intestinal Pathology in Goats Challenged with <i>Clostridium</i> perfringens Type D Wild-Type Strain CN1020 and its Genetically Modified Derivatives Morrell, E.L.;* Garcia, J.P.; Beingesser, J.; Adams, V.; Rood, J.I.; Uzal, F.A.	109
Comparison of Penicillin-Binding Proteins in Beta-Lactam Resistant Strains of <i>Clostridium perfringens</i> Park, M.;* Rafii, F.	110
Fluoroquinolone Resistance Selection Affects Sugar Transport Osmotolerance of Clostridium perfringens Park M · Rafii F *	111

Poster Index



	GUT MICROBIOME	
PI-26	Determination of the Effect of Maillard Products on the Taxonomic Composition on the Gut Microbiota	116
	ALJahdali, N.;* Gadonna, P.; Anton-Gay, P.; Carbonero, F.	
PI-27	Bacteroidetes and Firmicutes Numbers in Gut Microbiota of Adult Type 1 Diabetes Patients and Healthy Turkish People: Effects on Hosts TLR2 & TLR4 Gene Expression Levels	117
	Demirci, M.; Temeloglu-Keskin, E.; Cagatay, P.; Taner, Z.; Ozyazar, M.; Kocazeybek, B.; Kiraz, N.; Bahar-Tokman, H.*	
PI-28	The Mosaic of Cytochromes Expression from Bacteria to Man and Inflammation Processes	118
	Stavropoulou, E.;* Bezirtzoglou, E.	
PI-29	The Microbiota in a Model of Alzheimer's Disease, Aging, and Dietary Intervention	119
	Cox, L.M.;* Schafer, M.J.; Sohn, J.; Weiner, H.L.; Ginsberg, S.D.; Blaser, M.J.	
PI-30	The Fecal Microbiome of Dogs with Exocrine Pancreatic Insufficiency	120
	Isaiah, A.;* Parambeth, J.C.; Steiner, J.M.; Suchodolski, J.S.	
PI-31	Establishment and Development of Intestinal Microbiota in Preterm Infants of a Lebanese Tertiary Hospital	121
	Itani, T.;* Ayoub Moubareck, C.; Melki, I.; Delannoy, J.; Mangin, I.; Butel, M.J.; Karam Sarkis, D.	
PI-32	Characterization of Phenotypic and Genetic Diversity among Lachnospiraceae Isolates from the Human Gut Microbiota	122
	Lau, J.T.;* Surette, M.G.	
PI-33	Gut Microbiota in Healthy Subjects and Inpatients with Clostridium difficile Infection	123
	Flecher, T.B.; Miranda, K.R.;* Secco, D.A.; Peixoto, R.S.; Rosado, A.S.; do Carmo, F.L.; de Jesus, H.E.; Antunes, L.C.M.; de Paula, G.R.; Domingues, R.M.C.P.	
PI-34	Effect of Topsoil Exposure During Lactation on Subsequent Performance and Gut Microbiota in Pigs Vo, N.;* Tsai, T.; Kim, H.; Sales, M.A.; Wang, X.; Erf, G.E.; Kegley, E.B.; van der Merwe, M.; Buddington, R.; Maxwell, C.V.; Carbonero, F.	124
	, * .	

Reclassification of Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme, and Clostridium saccharogumia to a

Comparison of Growth of Clostridium perfringens on Different

Saavedra, L.;* Finegold, S.M.; Lawson, P.A.

Saleem, G.;* Sparks, N.; Houdijk, J.

Novel Genus, Flintia gen. nov.

in vitro Digested Broiler Diets



	STUDENT PRESENTATION POSTERS	
SP-1	The Enzymes of <i>Fusobacterium</i> spp. Involved in Hydrogen Sulfide Production from L-Cysteine <i>Basic, A.;</i> * <i>Blomqvist, M.; Dahlén, G.; Svensäter, G.</i>	126
SP-2	Re-examining the Germination Phenotypes of Several <i>C. difficile</i> Strains Bhattacharjee, D.;* Francis, M.B.; Ding, X.; McAllister, K.M.; Shrestha, R.; Sorg, J.A.	127
SP-3	Relationship Between Host Innate Immune Genetic Variation, Bacterial Vaginosis, Bacterial Colonization, and Cervicovaginal Cytokine Concentrations dela Cruz, E.J.;* Hawn, T.R.; Wiser, A.; Fredricks, D.N.; Marrazzo, J.M.	128
SP-4	Clostridium difficile Colonizes Alternative Nutrient Niches Across Susceptible Gut Community Structures Jenior, M.L.;* Leslie, J.L.; Schloss, P.D.	129
SP-5	Characterization of a Candidate Heme Detoxicification Operon in Clostridium difficile Knippel, R.J.;* Zackular, J.P.; Moore, J.L.; Skaar, E.P.	130
SP-6	Collagen-Like Protein BclA3 in <i>Clostridium difficile</i> Spores Influence the Adherence to Epithelial Caco-2 Cells Pizarro-Guajardo, M.;* Brito-Silva, C.; Kuehne, S.; Minton, N.P.; Paredes-Sabja, D.	131
SP-7	Binding of <i>Clostridium difficile</i> to Extracelular Matrix Proteins Santos, M.G.C.;* Trindade, C.N.R.; Rocha-Azevedo, B.; Ferreira, E.O.; Domingues, R.M.C.P.	132
SP-8	Characterization of Clinically Relevant Genetically Tractable Clostridium difficile Strain R20291 in a Mouse Model Winston, J.A.;* Thanissery, R.; Montgomery, S.A.; Theriot, C.M.	133
SP-9	The Potential of Using Metabonomic Approaches to Studying the Human Gut Microbiome Yen, S.,* Aucoin, M.G.; Allen-Vercoe, E.	134
	ANAEROBES IN THE MOUTH	
PII-1	Oral Anaerobes of the Dorsum of the Tongue: An Experimental Study Mailharin, A.; Saint-Marc, M.; Badet, C.*	136
PII-2	Implementation of Novel Species Identification into the Medical Microbiology Routine Diagnostics Workflow and Description of <i>Prevotella Colorescens</i> sp. nov. and <i>Prevotella Festinatalis</i> sp. nov. <i>Buhl, M.;* Willmann, M.; Oberhettinger, P.; Liese, J.; Autenrieth, I.B.; Marschal, M.</i>	137

Poster Index



PII-3	Predominant Bacterial Pathogens in Odontogenic Infections Egwari, L.O.;* Nwokoye, N.N.; Olubi, O.O.	138
PII-4	Three Variants of the Leukotoxin Gene in Human Isolates of Fusobacterium necrophorum Subspecies Funduliforme Holm, K.;* Collin, M.; Hagelskjaer-Kristensen, L.; Jensen, A.;	139
PII-5	Rasmussen, M. Antimicrobial Susceptibilities of Infrequent Prevotella Strains from Saliva	140
	Könönen, E.;* Lehto, L.; Gürsoy, M.	
PII-6	Antimicrobial Susceptibilites of Salivary Prevotella melaninogenica, Prevotella histicola, and Prevotella jejuni	141
	Lehto, L.;* Gürsoy, M.; Könönen, E.	
PII-7	Recognition of Laminin by Pathogenic Oral <i>Prevotella ssp Marre, A.T.O.;* Boente, R.F.; Ferreira, E.O.; Domingues, R.M.C.P.; Lobo, L.A.</i>	142
PII-8	Improvement of an Experimental Model of Oral Biofilm Nguyen, D.;* Badet, C.	143
PII-9	Functional Genomics in an Oral Campylobacter, Campylobacter rectus	144
	Threadgill, D.S.;* Conley, B.A.; Harrell, E.A.	
PII-10	Detection of <i>Fusobacterium necrophorum</i> from Pediatric Patient Throat Cultures by Selective Anaerobic Medium <i>Van, T.T.;* Cox, L.M.; Cox, M.E.; Dien Bard, J.</i>	145
	ANAEROBES IN THE GENITAL TRACT	
PII-11	Vaginal Microbiotas of Mother-Daughter Pairs Bassis, C.M.;* Alaniz, V.I.; Sack, D.E.; Bullock, K.A.; Lynn, C.S.; Quint, E.H.; Young, V.B.; Bell, J.D.	148
PII-12	Antimicrobial Resistance Rates of <i>Prevotella intermedia</i> and	
111 12	Related Species Isolated from Saliva of Post-Partum Women 10 Years Apart	149
	Gürsoy, M.;* Lehto, L.; Könönen, E.	
PII-13	Pigtail Macaque Vaginal Microbiota Characterized by Culture and Culture-Independent Sequencing	150
	DI IVAD II DA NA DE N	
	Rabe, L.K.;* Ravel, J.; Patton, D.L.; Ma, B.; Gajer, P.; Cosgrove Sweeney, Y.T.; Hillier, S.L.	
PII-14		151



	ANAEROBIC MICROBIOLOGY	
PII-15	Trends in Antimicrobial Resistance among Select Anaerobes from Sterile Sources over a 5-Year Period at an Academic Medical Center	154
	Bourdas, D.;* Hanlon, A.; Tekle, T.; Wakefield, T.; Harris, R.; Simner, P.; Carroll, K.C.	
PII-16	Testing an Antioxidants-Rich Medium to Grow Oral Anaerobes Aerobically	155
	Lange, E.; Melzer-Krick, B.; Henne, K.; Conrads, G.*	
PII-17	The International Anaerobe Quality Assurance Scheme (2)	156
	Copsey-Mawer, S.D.;* Morris, T.E.; Hughes, H.	
PII-18	Survival of Vaginal Microorganisms in Three Commercially Available Transport Systems	157
	DeMarco, A.L.;* Rabe, L.K.; Stoner, K.A.; Austin, M.N.; Avolia, H.A.; Gould, V.A.; Bracken, S.A.; Goldman, J.A.; Hillier, S.L.	
PII-19	Performance of Two Blood Culture Systems to Detect Anaerobic Bacteria: What is the Difference?	158
	Jeverica, S.;* Mueller-Premru, M.; Lampe, T.; Pišek, A.; Nagy, E.	
PII-20	Direct Identification of Anaerobic Bacteria from Positive Blood Culture Bottles Using the Sepsytiper Kit	159
	Mueller-Premru, M.; Jeverica, S.;* Lampe, T.; Pišek, A.; Kostrzewa, M.; Nagy, E.	
PII-21	Host – Pathogens Cross-Talk and the Influence of Host Stress Hormones on Bacterial Virulence <i>Lazar</i> , V.*	160
PII-22	Characterization of the Transcriptional Regulator BmoR in the Oxidative Stress Response of <i>Bacteroides fragilis</i>	161
	Teixeira, F.L.;* Pauer, H.; Domingues, R.M.C.P.; Rocha, E.R.; Lobo, L.A.	
PII-23	Novel Inhibitory Interactions between Prevotella and Streptococcu Species in the Cystic Fibrosis Lung: <i>in silico</i> Informing <i>in vitro</i>	s 162
	Whelan, F.J.;* Waddell, B.; Syed, S.A.; Rabin, H.; Parkins, M.D.; Surette, M.G.	
	PROBIOTICS	
PII-24	Inhibitory Effect of the Probiotic <i>Lactobacillus delbrueckii</i> subsp. <i>Bulgaricus</i> G-LB-44 on Pathogenic Organisms Grown in Organic Juice and Produce	164
	DuBois, A.M.;* Delaney, M.L.; DuBois, G.M.; Onderdonk, A.B.	

Poster Index



PII-25	Lactobacillus reuteri Decreases Adherent Tumor Mucins and Enhances Chemotheraputic Susceptibility	165
	Engevik, M.A.;* Luk, B.K.; Ganesh, B.P.; Hall, A.; Versalovic, J.	
PII-26	Probiotics and C. Difficile Infection (CDI)	166
	Goldstein, E.J.C.;* Johnson, S.J.; Louie, T.J.; Maziade, P-J.; Millette, M.; Sniffen, J.C.	
PII-27	Theoretical Aspects for Practical Applications of Intestinal <i>Lactobacillus</i> Species Strains	167
	Mikelsaar, M.;* Štšepetova, J.; Mändar, R.; Songisepp, E.; Sepp, E.	
PII-28	Bio-K+ Probiotic Strains Reduce Toxin and Spore-Forming Gene Expression in <i>Clostridium difficile</i>	168
	Paquette, P.; Frappier, M.; Auclair, J.; Gunaratnam, S.; Millette, M.*	
PII-29	<i>In vitro</i> Investigations of Innovative Fructooligosaccharides: Modulations of Probiotics, Enteropathogens and Colic Cell Proliferation	169
	Grimoud, J.; Ouarné, F.; Gignac-Brassard S.; Roques, C.*	
PII-30	Isolation of Lactic Acid Bacteria from Oysters (<i>Crassostrea gigas</i>) for Their Potential Use as Probiotics	170
	Kang, C.H.; Shin, Y.J.; Kim, W.R.; Jang, S.C.; Gu, T.; Jung, Y.; So, J.S.*	
PII-31	New Functional Foods with Probiotics and Prebiotics: Perspectives in the Control of Obesity	171
	Torriani, S.*	
	CLOSTRIDIUM DIFFICILE: PATHOGENESIS	
PII-32	The Role of Flagella in <i>Clostridium difficile</i> Pathogenesis: Comparison Between a Non-Epidemic and an Epidemic Strain <i>Baban, S.T.;* Kuehne, S.A.; Barketi-Klai, A.; Cartman, S.T.;</i>	175
	Kelly, M.L.; Hardie, K.R.; Kansau, I.; Collignon, A.; Minton, N.P.	
PII-33	Assessment of Additional Virulence Factors Present in the <i>in vitro</i> Supernatant of <i>Clostridium difficile</i>	176
	Castro-Peña, C.;* López-Ureña, D.; Rodríguez, C.; Quesada-Gómez, C.; Chaves-Olarte, E.	
PII-34	Correlation of Binary Toxin with Clinical Outcomes in <i>C. Difficile</i> Infection (CDI)	177
	Cihlar S.;* Siddiqui, F.; Cheknis, A.; Sambol, S.P.; Carman, R.; Lyerly, M.; Gerding, D.N.; Johnson, S.	
PII-35	Mucin-Associated Bacterial Communities During <i>Clostridium difficile</i> Infection	178
	Semenyuk, E.G.; Ashraf, A.; Poroyko, V.A.; Johnston, P.F.; Knight, K.L.; Gerding, D.N.; Driks, A.*	



Poster Index



_				
PII-36	A Novel Negative Regulator of Sporulation Initiation in Clostridium difficile	179	PIII-4	Molecular Epidemiology of <i>Clostridium difficile</i> Isolated in the United States, 2014
	Edwards, A.N.;* Childress, K.O.; McBride, S.M.			Karlsson, M.;* Paulick, A.; Albrecht, V.; Granade, M.; Guh, A.;
PII-37	Antigenic Differentiation of Toxigenic and Non-Toxigenic Strains of Clostridium difficile	180	PIII-5	Rasheed, J.K. Clostridium difficile Infection Surveillance in a Tertiary Medical
	Gowrishankar, R.;* Williamson, Y.M.; Kirkham, H.; Barr, J.R. Moura, H.			Center Reveals Significant Strain Variation Mansoor, A.;* Shehab, K.; Anwar, F.; Viswanathan, V.K.;
PII-38	Structural Studies of the <i>Clostridium difficile</i> toxin TcdA in Complex with a Neutralizing Monoclonal Antibody	181	PIII-6	Vedantam, G. Occurrence of Clostridium difficile Infections in Hospitals of
	Kroh, H.K.;* Chandrasekaran, R.; Ohi, M.D.; Nyborg, A.C.; Rainey, J.; Warrener, P.; Spiller, B.W.; Lacy, D.B.		S	Silesia, Poland Aptekorz, M.; Szczegielniak, A.; Harmanus, C.; Kuijper, E.; Martirosian, G.*
PII-39	Extracellular Vesicles of Clostridium difficile	182	PIII-7	The Role of Ribotype 106 as a Cause of Clostridium difficile
	Lopes, A.S.; Silva, R.C.; Boente, R.F.; Domingues, R.D.P.; Miranda, K.R.; Lobo, L.A.*		1 111-7	Infection in the United States, 2012-2014
PII-40	Proteomic Analysis of the Exosporium (Spores) of Brazilian <i>Clostidium difficile</i> Ribotypes Treated with Hospital Antibiotics	183		Paulick, A.;* Karlsson, M.; Albrecht, V.; Granade, M.; Guh, A.; Rasheed, J.K.; Limbago, B.; EIP CDI Pathogen Group
	Motta, K.O.L.S.; Trindade, C.N.R.; Ferreira, T.G.; Miyajima, F.; Domingues, R.M.C.P.; Ferreira, E.O.*	PIII-8	Identification and Characterization of <i>Clostridium difficile</i> Strains Isolated from Dog Stools in Rio De Janeiro, Brazil	
PII-41	Metaproteomics Unveils the Fate of Clostridium difficile Toxins in	104		Rainha, K.; Fernandes, R.F.; Miyajima, F.; Roberts, P.; Santos, J.; Domingues, R.M.C.P.; Ferreira, E.O.*
	Stool Samples Moura, H.;* Kraft, C.S.; Williamson, Y.M.; Kirkham, H.;	184	PIII-9	Clostridium difficile Ribotype 027 is an Independent Risk Factor for Recurrent C. difficile Infection
DII 42	Gowrishankar, R.; Barr, J.R.			Rao, K.;* Young, V.B.
PII-42	Exploring the Role of an Alanine Racemase during <i>Clostridium difficile</i> Spore Germination	185	PIII-10	Characterization of <i>Clostridium difficile</i> Strains Isolated from Immunocompromised Patients in Brazil
PII-43	Shrestha, R.;* Sorg, J.A. TPL-2 is a Key Regulator of Inflammation in C. difficile Infection	186		Secco, D.A.; Boente, R.F.; Miranda, K.R.; Santos-Filho, J.; Miyajima, F.; Nouer, S.A.; Domingues, R.M.C.P.*
	Wang, Yu.; Ju, X.; Tzipori, S.; Feng, H.; Greenberg, A.; Sun, X.*		PIII-11	Canine Pets are a Potential Source of Community Acquired
PII-44	Inhibition of Spore Germination by Microbial Derived Secondary Bile Acids in <i>Clostridium difficle</i> Strains that Vary in Ribotype	187		Clostridium difficile Infection in Humans Stone, N.E.;* Sidak-Loftis, L.C.; Sahl, J.W.; Vazquez, A.J.;
	Thanissery, R.S.;* Theriot, C.M.			Busch, J.D.; Keim, P.; Wagner, D.M.
	CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY		PIII-12	Ribotype 017 among Toxigenic Isolates are Predominant Toxigenic <i>C. difficile</i> in Southern Taiwan
PIII-1	Ribotype Diversity of Recent Clinical Clostridium difficile			Hung, Y.P.; Tsai, B.Y.; Ko, W.C.; Tsai, P.J.*
		191	PIII-13	Colonization of Toxigenic <i>Clostridium difficile</i> among ICU Patients: A Prospective Study
	Kuo, J.; Garey, K.W.			Zhang, X.; Wang, X.; Yang, J.; Zong, Z.*
PIII-2	High Colonization of <i>Clostridium difficile</i> among Different Nigerian Age Groups; Possible Reason for Low Incidence of CDI	192		CLOSTRIDIUM DIFFICILE: MICROBIOLOGY
	Egwuatu, T.O.;* Ogunsola, F.T.; Olalekan, A.O.; Egwuatu, C.A.		PIII-14	Nonsteroidal Anti-Inflammatory Drugs Alter the Gut Microbiome
PIII-3	Emergence of a New <i>C. difficile</i> Strain, REA Group DQ, Related to REA Group BI/Ribotype 027 <i>Johnson, S.;* Petrella, L.; Siddiqui, F.; Sambol S.P.;</i>	193		and Increase the Severity of <i>Clostridium difficile</i> Infection in Mice <i>Trindade, B.C.; Kirk, L.; Rogers, L.M.; Zackular, J.P.; Skaar, E.P.; Schloss, P.D.; Lyras, D.; Maseda, D.; Crofford, L.J.; Aronoff, D.M.</i> *

M.; Zackular, J.P.; Skaar, E.P.; D.; Crofford, L.J.; Aronoff, D.M.*

Gulvik, C.; Limbago, B.; Gerding, D.N.; Donskey, C.J.



Poster Index



PIII-15	Design of a Two-Plex Assay for Detection of <i>Clostridium difficile</i> Toxins A and B	208	PIII-27	Comparative Exoproteome of <i>Clostridium difficile</i> Brazilian Ribotypes Treated with Subinhibitory Concentrations of Antibiotics	220
	Banz, A.;* Riou, B.; Lantz, A.; Foussadier, A.			Trindade, C.N.R.;* Moura, H.; Barr, J.R.; Miyajima, F.; Ferreira, T.G.; Ferreira, E.O.; Domingues, R.M.C.P.	
PIII-16	In vitro Activity of Cadazolid and Eight Comparator Antimicrobial Agents against 50 Isolates of Clostridium difficile, 379 Other Anaerobes and 174 Aerobic Organisms	209		CLOSTRIDIUM DIFFICILE: MANAGEMENT	
	Citron, D.M.;* Tyrrell, K.L.; Goldstein, E.J.C.		PIII-28	Impact of Surotomycin Treatment against Clostridium difficile	223
PIII-17	Toxin Profiles, PCR Ribotypes and Resistance Patterns of <i>Clostridium difficile</i> : A Multicenter Study in China, 2012-2013	210		Bassères, E.;* Endres, B.T.; Khaleduzzaman, M.; Miraftabi, F.; Alam, M.J.; Chesnel, L.; Garey, K.W.	
	Gao, Q.; Wu, S.; Huang, H.;* Ni, Y.; Chen, Y.; Hu, Y.; Yu, Y.		PIII-29	Vancomycin-Resistant Enterococcus (VRE) Colonization and	
PIII-18	Colonization of Binary Toxin-Positive and Binary Toxin-Negative Clostridium difficile Strains in Hamsters	211		C. difficile Epidemiology: The Deflect-1 Study Chesnel, L.; Devaris, D.; Citron, D.M.; Sambol, S.P.; Hecht, D.;	224
	Johnston, P.F.;* Semenyuk, E.; Siddiqui, F.; Sambol, S.P.;			Sears, P.	
PIII-19	Driks, A.; Gerding, D.N.; Johnson, S. Characterisation of Random Clostridium difficile Mutants Less		PIII-30	Stability of Three Oral Vancomycin (VA) Preparations Stored at 2-5°C and Ambient Temperatures for Up to 60 Days	225
	Susceptible to Ridinilazole (SMT19969) in Terms of Fitness Cost	212		Citron, D.M.;* Tyrrell, K.L.; Renaud, K.; Goldstein, E.J.C.	
	Budd, P.; Kelly, M.L.; Vickers, R.J.; Winzer, K.; Minton, N.P.; Kuehne, S.A.*		PIII-31	Morphologic Changes with Antibiotics Targeting <i>Clostridium difficile</i> Provide Novel Insight for Drug Mechanism of Action	226
PIII-20	In vitro Susceptibility and Genotyping of 1776 Pre-Treatment Isolates of Clostridium difficile Recovered from a Global Clinical	212		Endres, B.T.;* Bassères, E.; Memariani, A.; Chang, L.; Alam, M.J.; Kakadiaris, I.A.; Chesnel, L.; Garey, K.W.	
	Trial Merriam, C.V.;* Citron, D.M.; Sambol, S.P.; Wilcox, M.B.; Goldstein, E.J.C.; Dorr, M.B.	213	PIII-32	Recurrent Clostridium difficile Infection and Colonization in the 12 Months following Administration of Actoxumab and Bezlotoxumab	227
PIII-21	Agar Dilution versus Broth Microdilution Methods for Antimicrobial Susceptibility Testing of Clostridium difficile Nary, J.;* Citron, D.M.; Chesnel, L.; Dale, S.E.	214		Goldstein, E.J.C.;* Citron, D.M.; Gerding, D.N.; Wilcox, M.H.; Tipping, R.; Dorr, M.B.; Gabryelski, L.; Eves, K.; Kartsonis, N.; Pedley, A.	
PIII-22	Exploring the Diversity of Toxinotype V <i>Clostridium difficile</i> Strains through Whole Genome Sequencing <i>Norman, K.N.;* Scott, H.M.</i>	215	PIII-33	SYN-010, a Novel, Modified-Release Formulation of Lovastatin Lactone Designed to Reduce Methane Production by Methanobrevibacter smithii and thus Relieve Symptoms of	
PIII-23	Benefits of <i>Clostridium difficile</i> Polymerase Chain Reaction Screening Followed by Toxin Confirmation <i>Robinson</i> , <i>P.</i> *	216		Irritable Bowel Syndrome with Constipation Kokai-Kun, J.F.;* McFall, H.; Coughlin, O.; Rezaie, A.; Wacher, V.; Pimentel, M.; Gottlieb, K.; Sliman, J.	228
PIII-24	Analysis of Surface Proteins Obtained from Clostridium Difficile		PIII-34	A Combination of the Probiotic and Prebiotic Prevents the	
1111 21	Brazilian Strains Cultivated with Antibiotics	217		Germination of Clostridium Difficile Spores	229
	Ferreira, T.G.; Moura, H.; Barr, J.H.; Miyajima, F.; Domingues, R.M.C.P.; Ferreira, E.O.*			Rätsep, M.; Kõljalg, S.; Sepp, E.; Smidt, I.; Truusalu, K.; Songisepp, E.; Naaber, P.; Mikelsaar, M.*	
PIII-25	SMT 19969: A Novel Agent for <i>Clostridium difficile</i> Infection (CDI): Synopsis of Microbiology & Phase 1 Study	218	PIII-35	A Quaternary Ammonium Solution Containing Chemical Sensitizers Induces Susceptibilty in <i>Clostridium difficile</i> Spores to Ambient Environmental Stressors	230
	Vickers, R.J.; Corbett, D.; Warn, P.; Citron, D.M.; Goldstein, E.J.C.; Best, E.; Wilcox, M.; Tillotson, G.S.*			Nerandzic, M.M.;* Donskey, C.J.	230
PIII-26	In vivo Efficacy of SMT 19969, Vancomycin, and Fidaxomicin in a	210	PIII-36	CDAD-Daysyms®: A New Patient-Reported Outcome Tool for	221
	Hamster Model of CDI Teague, J.; Wise, A.; Thommes, P.; Burgess, E.; Daws, G.; Payne L.J.; Vickers, R.J.; Warn, P.; Tillotson, G.S.*	219		Clostridium difficile-associated Diarrhea (CDAD) Kleinman, L.; Talbot, G.H.; Schüler, R.; Broderick, K.; Revicki, D.; Nord, C.E.*	231



Author Index



PIII-37	Molecular Basis for Neutralization of <i>Clostridium difficile</i> Toxins by the Antitoxin Antibodies Actoxumab and Bezlotoxumab	232
	Hernandez, L.D.; Kroh, H.K.; Beaumont, M.; Sheth, P.R.; Yang, X.; DiNunzio, E.; Rutherford, S.A.; Ohi, M.D.; Murgolo, N.J.; Xiao, L.; Orth, P.; Racine, F.; Reichert, P.; Hsieh, E.; Ermakov, G.; Strickland, C.; Lacy, D.B.; Therien A.G.*	
PIII-38	Clostridium difficile: The Holistic Costs in the Era of New	222
	Therapies Tillotson, J.;* Chopra, T.; Tillotson, G.S.	233
PIII-39	Ridinilazole (RDZ) for <i>Clostridium difficile</i> Infection (CDI):	
1111 07	Results from the Codify Phase 2 Clinical Trial	234
	Vickers, R.J.; Wilcox, M.H.; Gerding, D.N.; Tillotson, G.S.*	
PIII-40	Determining the Stability of Frozen and Lyophilized Fecal Microbiota Transplant (FMT) Product Used to Treat Patients with Multiple Bouts of <i>Clostridium difficile</i> Infection (CDI)	235
	Valilis, E.M.;* Jiang, Z.D.; Ke, S.; DuPont, H.L.	
PIII-41	Assessment of Efficacy of Bezlotoxumab for Prevention of Clostridium difficile Infection Recurrence by Diagnostic Test Method Wilcox, M.H.;* Rahav, G.; Dubberke, E.; Gabryelski, L.; Eves, K.; Tipping, R.; Guris, D.; Kartsonis, N.; Dorr, M.B.	236

Adams, V.	109	Bezirtzoglou, E.	118
Agung, I.G.	84	Bhattacharjee, D.	60, 127
Ajami, N.J.	28	Bilverstone, T.	62
Alam, M.J.	191, 223, 226	Blackburn, D.	92
Alaniz, V.I.	148	Blaser, M.J.	119
Albrecht, V.	194, 197	Blomqvist, M.	126
Aldape, M.J.	16	Boente, R.F.	142, 182, 200
ALJahdali, N.	116	Bouillaut, L.	83
Allen-Vercoe, E.	134	Bourdas, D.	154
Amadio, J.	191	Bracken, S.A.	157
Anton-Gay, P.	116	Bradshaw, M.	14
Antunes, L.C.M.	123	Briano, C.	15
Anwar, F.	195	Bristol, J.A.	51
Aptekorz, M.	196	Brito-Silva, C.	131
Aronoff, D.M.	207	Broderick, K.	231
Arinton, I.G.	84	Brown, B.	49
Ashraf, A.	178	Brown, J.C.S.	106
Auclair, J.	168	Bryant, A.E.	16
Aucoin, M.G.	134	Buddington, R.	124
Austin, M.N.	157	Buhl, M.	137
Autenrieth, I.B.	137	Bullock, K.A.	148
Avila-Campos, M.J.	13	Bunge, K.	57
Avolia, H.A.	157	Budd, P.	212
Ayepola, O.O.	89, 90	Burgess, E.	219
Ayoub Moubareck, C.	121	Burroughs, M.	49
,		Busch, J.D.	201
Babalola, F.	89	Butel, M.J.	121
Baban, S.T.	175		
Badet, C.	136, 143	Cagatay, P.	117
Bahar-Tokman, H.	91, 117	Calderón-Romero, P.	86
Bailey-Person, M.	95	Carbonero, F.	116, 124
Banz, A.	208	Carman, R.	177
Barketi-Klai, A.	175	Carroll, K.C.	154
Barr, J.H.	217	Cartman, S.T.	175
Barr, J.R.	14, 180, 184, 220	Castelpoggi, J.P.	12
Basic, A.	126	Castro-Peña, C.	176
Bassères, E.	223, 226	Celik, V.	91
Bassis, C.M.	148	Centor, R.M.	4
Bayer, C.R.	16	Chandrasekaran, R.	61, 181
Beamer, M.A.	57	Chang, L.	226
Beaumont, M.	232	Chappell, J.D.	82
Beingesser, J.	109	Chaves-Olarte, E.	176
Bell, J.D.	148	Cheknis, A.	177
Bellio, M.	12	Chen, Y.	210
Bernard, K.A.	43	Chesnel, L.	214, 223, 224, 226
Best, E.	218	Childress, K.O.	179
,			



Author Index



Charma T	233	Din a V	60 127
Chopra, T.		Ding, X.	60, 127
Christian, L.M.	32	DiNunzio, E.	232
Chukwu, E.E.	13	Dixon, R.A.	106
Cihlar S.	177	do Carmo, F.L.	123
Citron, D.M.	209, 213, 214, 218, 224,	Domingues, R.D.	
Cl 1 C 4	225, 227	Domingues, R.M.	
Clark, C.A.	114		1, 183, 198, 200, 217, 220
Claus, S.P.	19	Donskey, C.J.	10, 193, 230
Coker, A.O.	13	Dorr, M.B.	213, 227, 236
Collignon, A.	175	Dressendorfer, L.	
Collin, M.	139	Driks, A.	178, 211
Collins, D.A.	84	Dubberke, E.	69, 236
Conley, B.A.	144	DuBois, A.M.	164
Connelly, S.	51	DuBois, G.M.	164
Conrads, G.	37, 155	DuPont, H.L.	235
Cook, D.N.	26	Dutra, F.	15
Copsey-Mawer, S.	D. 156		
Corbett, D.	218	Edwards, A.N.	179
Cosgrove Sweeney	y, Y.T. 150	Edwards, K.M.	82
Coughlin, O.	228	Egwari, L.O.	89, 90, 93, 138
Coutinho-Silva, R.	12	Egwuatu, C.A.	192
Cox, L.M.	119, 145	Egwuatu, T.O.	192
Cox, M.E.	145	Elliott, B.	84
Crofford, L.J.	207	Endres, B.T.	223, 226
Cu-Uvin, S.	33	Engevik, M.A.	165
		Erf, G.E.	124
Dabdoub. S.M.	30, 31	Erickson, J.	49
Dahlén, G.	126	Ermakov, G.	232
Dal, F.	91	Eves, K.	227, 236
Dale, S.E.	214		
Darkoh, C.	76	Faparusi, F.	90
DaSilva, W.L.	114	Faparusi, J.	89
Daws, G.	219	Feng, H.	186
de Almeida, B.J.	12	Fernandes, R.F.	198
de Jesus, H.E.	123	Ferreira, E.O.	12 , 132, 142, 183, 198,
de Paula, G.R.	123		217, 220
Deepe Jr., G.	63	Ferreira, T.G.	183, 217, 220
dela Cruz, E.J.	128	Fichorova, R.N.	33, 151
Delaney, M.L.	151, 164	Finegold, S.M.	6, 112
Delannoy, J.	121	Flattery, A.	85
DeLong, A.K.	33	Flecher, T.B.	123
DeMarco, A.L.	157	Flynn, K.J.	94
Demirci, M.	91, 117	Foster, J.A.	46
Devaris, D.	224	Foussadier, A.	208
Dias, M.	12	Francis, M.B.	60, 127
Dien Bard, J.	145	Frappier, M.	168
		T I	

			\mathcal{L}
Fredricks, D.N.	128	Hardie, K.R.	175
Freedman, J.C.	107	Harmanus, C.	196
Fujita, J.	108	Harrell, E.A.	92, 144
Furlan-Freguia, C.	51	Harris, R.	154
		Hasan, N.A.	51
Gabryelski, L.	227, 236	Hassan, S.A.	84
Gadonna, P.	116	Hawn, T.R.	128
Gajer, P.	150	Hayashi, M.	96
Ganesan, S.M.	30, 31	Hecht, D.	224
Ganesh, B.P.	165	Henne, K.	155
Gao, Q.	210	Hernandez, L.D.	85, 232
Garcia, J.P.	109	Hillier S.L.	54, 57, 150, 157
Garey, K.W.	191, 223, 226	Hirai, J.	108
Garg, S.	49	Holder, M.E.	28
Gasem, M.H.	84	Holm, K.	139
Genco, C.A.	38	Horz, H.P.	37
Gerding, D.N.	68, 177, 178, 193, 211,	Houdijk, J.	113
•	227, 234	Hsieh, E.	232
Ghose-Paul, C.	67	Hu, Y.	210
Gignac-Brassard S.	169	Huang, H.	210
Ginsberg, S.D.	119	Hubert, S.	51
Goksoy, E.	91	Hughes, H.	156
Goldman, J.A.	157	Hung, Y.P.	202
Goldstein, E.J.C.	7, 166, 209, 213, 218,		
	225, 227	Isaiah, A.	120
Gonzalez, C.	100	Itani, T.	121
Gottlieb, K.	228		
Gould, V.A.	157	Jamieson, D.J.	33
Gowrishankar, R.	180, 184	Jang, S.C.	170
Granade, M.	194, 197	Jenior, M.L.	64, 129
Greenberg, A.	186	Jensen, A.	139
Grimoud, J.	169	Jeverica, S.	97, 158,159
Gu, T.	170	Jiang, Z.D.	235
Guh, A.	194, 197	Johnson, E.A.	14
Gulvik, C.	193	Johnson, S.	66, 166, 177, 193, 211
Gunaratnam, S.	168	Johnston, P.F.	178, 211
Gunaydın, M.	91	Ju, X.	83, 186
Guris, D.	236	Junaid, D.	151
Gürsoy, M.	140, 141, 149	Jung, Y.	170
Habibie, T.	84	Kakadiaris, I.A.	226
Hackel, M.	95	Kalb, S.	14
Hagelskjaer-Krister	sen, L. 139	Kaleko, M.	51
Hall, A.	165	Kang, C.H.	170
Handrianto, P.	84	Kansau, I.	175
Hanlon, A.	154	Karam Sarkis, D.	121



Author Index



Karlsson, M.	194, 197	Lazar, V.	160
Kartsonis, N.	227, 236	Leblebicioglu, B.	32
Ke, S.	235	Lee, C.H.	69
Kegley, E.B.	124	Lee, Y.Y.	84
Keim, P.	201	Lehto, L.	140, 141, 149
Kelly, C.	48	Leister-Tebbe, H.	95
Kelly, M.L.	175, 212	LeRoy, C.I.	19
Khaleduzzaman, M.	223	Leslie, J.L.	64, 129
Khanna, S.	69	Lewis, D.E.	28
Kim, H.	124	Liese, J.	137
Kim, W.R.	170	Limbago, B.	193, 197
Kinder, M.N.	92	Lin, G-L.	14
King, C.C.	33	Little, J.L.	82
Kinjo, T.	108	Llanco, L.A.	13
Kinsmore, N.L.	62	Lobo, L.A.	12 , 142, 161, 182
Kiratisin, P.	84	Lombardo, M-J.	26
Kiraz, N.	91, 117	Lopes, A.S.	182
Kirk, L.	207	López-Ureña, D.	176
Kirkham, H.	180, 184	Louie, T.J.	166
Klein, R.S.	33	Luk, B.K.	165
Kleinman, L.	231	Luu, N.	151
Knight, K.L.	178	Lyerly, M.	177
Knippel, R.J.	130	Lynn, C.S.	148
Ko, W.C.	202	Lyras, D.	78, 207
Kocazeybek, B.	91, 117	•	
Koizumi, Y.	101, 102	Ma, B.	150
Kõljalg, S.	229	Mackay, C.R.	12
Kokai-Kun, J.F.	228	Madan, R.	63
Konganti, K.	92	Mailharin, A.	136
Könönen, E.	140, 141, 149	Mallozzi, M.J.G.	28
Kostrzewa, M.	159	Mändar, R.	167
Kraft, C.S.	184	Mangin, I.	121
Kroh, H.K.	181, 232	Mansoor, A.	195
Kucuk, Y.	91	Mariño, E.	12
Kuehne, S.A.	62, 131, 175, 212	Marrazzo, J.M.	128
Kumar, P.S.	30, 31, 31, 39	Marre, A.T.O.	142
Kuijper, E.J.	73, 196	Marschal, M.	137
Kuo, J.	191	Martirosian, G.	196
		Maseda, D.	207
La Ragione, R.M.	19	Maslanka, S.	14
Lacy, D.B.	61, 181, 232	Maxwell, C.V.	124
Lampe, T.	158, 159	Mayer, K.H.	33
Lange, E.	155	Maziade, P-J.	166
Lantz, A.	208	McAllister, K.N.	60, 127
Lau, J.T.	122	McBride, S.M.	179
Lawson, P.A.	27, 42, 112	McClane, B.A.	107

McFall, H.	228	Oberhettinger, P.	137
McPherson, J.	191	Ogunsola, F.T.	13, 192
Melki, I.	121	Ohi, M.D.	181, 232
Melzer-Krick, B.	155	Olalekan, A.O.	192
Memariani, A.	226	Olguín-Araneda, V.	86
Merriam, C.V.	213	Oliveira, A.C	12
Mete, B.	91	Olubi, O.O.	89, 90, 93, 138
Meyn, L.A.	57	Onderdonk, A.B.	151, 164
Mikamo, H.	101, 102, 108	Orenstein, R.	69
Mikelsaar, M.	167, 229	Orth, P.	232
Milano-Céspedes,	M. 86	Ouarné, F.	169
Millette, M.	166, 168	Ozyazar, M.	117
Minton, N.P.	62, 131, 175, 212		
Miraftabi, F.	223	Pamulapati, S.	30
Miranda, J.	191	Paquette, P.	168
Miranda, K.R.	123, 182, 200	Paredes-Sabja, D.	86
Miyajima, F.	183, 198, 200, 217, 220	Parambeth, J.C.	120
Montgomery, S.A.	133	Paredes-Sabja, D.	131
Moore, J.L.	130	Park, M.	110, 111
Moura, H.	217, 220	Parkins, M.D.	162
Morrell, E.L.	109	Paropkari, A.D.	32
Morris, T.E.	156	Patel, N.B.	27
Motta, K.O.L.S.	183	Patton, D.L.	150
Moura, H.	180, 184	Pauer, H.	161
Mueller-Premru, N	•	Paulick, A.	194, 197
Murgolo, N.J.	232	Payne D.C.	82
N. I. D.	220	Payne L.J.	219
Naaber, P.	229	Pedley, A.	227
Nadiah, H.Z.	84	Peeler-Fletcher, S.A.	92
Nagy, E.	36, 97, 98, 158, 159	Peixoto, R.S.	123
Nary, J.	214	Pellet, S.	14
Navarro, M.	15	Perry, J.D.	97
Nawrocki, E.M.	14	Persiani, M.	15
Nerandzic, M.M.	230	Petrella, L.	193 63
Nguyen, D.	143 210	Petri Jr., W.A.	114
Ni, Y. Nicholson, M.R.	82	Pettis, G.S. Petrosino, J.F.	28
Nishiyama, N.	101, 102	Pier, C.L.	14
Nord, C.E.	72, 231	Pimentel, M.	228
Norman, K.N.	215	Pišek, A.	158, 159
Nouer, S.A.	200	Pizarro-Guajardo, M.	130, 133
Nwokoye, N.N.	89, 90, 93, 138	Plaza-Garrido, Á.	86
Nwaokorie, F.O.	13	Poroyko, V.A.	178
Nyborg, A.C.	181	Putsathit, P.	84
ryporg, A.C.	101	Pyles, R.B.	56
		i yics, K.D.	30



Author Index



Quesada-Gómez, C.	176	Sales, M.A.	124	Srinivasan, S.	55	Urbán, E.	97, 98
Quint, E.H.	148	Sambol, S.P.	177, 193, 211, 213, 224	Stavropoulou, E.	118	Uzal, F.A.	15, 109
		Sankaranarayanar	n, K. 27	Steiner, J.M.	120		
Rabe, L.K.	150, 157	Santos-Filho, J.	200	Stevens, D.L.	16	Valilis, E.M.	235
Rabin, H.	162	Santos, J.	198	Stevenson, E.	62	Van, T.T.	145
Racine, F.	232	Santos, M.G.C.	132	Stone, N.E.	201	van der Merwe,	M. 124
Rafii, F.	110, 111	Sarker, M.R.	86	Stoner, K.A.	157	Van Horn, G.T.	82
Rahav, G.	236	Sattar, A.	85	Strickland, C.	232	Vazquez, A.J.	201
Rainey, F.A.	42	Schafer, M.J.	119	Štšepetova, J.	167	Vedantam, G.	28, 77, 195
Rainey, J.	181	Scharfstein, J.	12	Subramanian, P.	51	Velasco, N.	100
Rainha, K.	198	Schloss, P.D.	94, 129, 207	Suematsu, H.	101, 102, 108	Velidedeoglu, M	I. 91
Ramos Jr., E.S.	12	Schmidt, T.M.	20	Suchodolski, J.S.	120	Vendrov, K.C.	50
Rasmussen, M.	139	Schüler, R.	231	Sun, X.	83, 186	Versalovic, J.	165
Rao, K.	199	Scott, H.M.	215	Surette, M.G.	47, 122, 162	Vickers, R.J.	212, 218, 219, 234
Rasheed, J.K.	194, 197	Sears, C.L.	22	Svensäter, G.	126	Vieira, L.Q.	12
Rätsep, M.	229	Sears, P.	224	Syed, S.A.	162	Vinjé, J.	82
Ravel, J.	150	Secco, D.A.	123, 200	Szczegielniak, A.	196	Viswanathan, V.	K. 195
Reichert, P.	232	Seekatz A.M.	50	· ·		Vlahov, D.	33
Renaud, K.	225	Semenyuk, E.G.	178	Talbot, G.H.	231	Vo, N.	124
Revicki, D.	231	Semenyuk, E.	211	Tanaka, K.	96		
Rezaie, A.	228	Sepp, E.	167, 229	Taner, Z.	91, 117	Wacher, V.	228
Rice, S.N.	16	Shehab, K.	195	Tang, Y.W.	82	Waddell, B.	162
Riley, T.V.	84	Sheth, P.R.	232	Teague, J.	219	Wagner, D.M.	201
Riou, B.	208	Shin, Y.J.	170	Teixeira, F.L.	161	Wakefield, T.	154
Roberts, P.	198	Shrestha, R.	60, 127, 185	Tekle, T.	154	Wang, X.	124, 203
Romero, A.	15	Sidak-Loftis, L.C.	201	Temeloglu-Keskin,	, E. 117	Wang, Y.	83
Robinson, P.	216	Siddiqui, F.	177, 193, 211	Tepp, W.H.	14	Wang, Y.	83, 186
Rocha, E.R.	161	Sieber, J.R.	20	Thanissery, R.S.	18, 133, 187	Warn, P.	85, 218, 219
Rocha-Azevedo, B.	132	Simner, P.	154	Therien, A.G.	85, 232	Warrener, P.	181
Rodríguez, C.	176	Silva, R.C.	182	Theriot, C.M.	18, 50, 133, 187	Weiner, H.L.	119
Rogers, L.M.	207	Skaar, E.P.	130, 207	Threadgill, D.S.	92, 144	Whelan, F.J.	162
Rood, J.I.	109	Sliman, J.	51, 228	Tierney, D.	97	Wilcox, M.H.	74, 213, 218, 227, 234, 236
Roques, C.	169	Smidt, I.	229	Thommes, P.	85, 219	Williamson, Y.M	I. 180, 184
Rosado, A.S.	123	Sniffen, J.C.	166	Tillotson, G.S.	23, 218, 219, 233, 234	Willmann, M.	137
Roxas, B.P.	28	So, J.S.	170	Tillotson. J.	233	Winston, J.A.	18, 133
Rumbaugh, K.P.	103, 104	Sobel, J.D.	33	Tipping, R.	227, 236	Winzer, K.	212
Rutherford, S.A.	232	Sohn, J.	119	Torriani, S.	171	Wise, A.	219
Ryan, S.	151	Sóki, J.	97, 98	Trindade, B.C.	207	Wiser, A.	128
		Solis, L.	13	Trindade, C.N.R.	132, 183, 220	Woodward, M.J.	. 19
Saavedra, L.	112	Solís, M.B.	100	Tsai, B.Y.	202	Wu, S.	210
Sack, D.E.	148	Solomkin, J.S.	5	Tsai, P.J.	202		
Sahl, J.W.	201	Sonenshein, A.L.	83	Tsai, T.	124	Xiao, L.	232
Sahm, D.	95	Songisepp, E.	167, 229	Truusalu, K.	229	Xue, J.	63
Saint-Marc, M.	136	Sorg, J.A.	60, 127, 185	Tyrrell, K.L.	209, 225		
Sakanashi, D.	108	Sparks, N.	113	Tzipori, S.	186		
Saleem, G.	113	Spiller, B.W.	181	_			
	2	54			21	55	



Yamagishi, Y.	101, 102, 108	Zackular, J.P.	79, 130, 207
Yamamoto, H.S.	33, 151	Zamboni, D.	12
Yang, J.	203	Zhang, X.	203
Yang, KT.	114	Zhang, Z.	85
Yang, X.	232	Zhao-Fleming, H.H.	103, 104
Yemisen, M.	91	Zhu, Y.	82
Yen, S.	134	Zong, Z.	203
Young, V.B.	50, 64, 148, 199	Zurita, J.	100
Yu, Y.	210		

Anaerobe



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

Editor-in-Chief

Professor E. Nagy University of Szeged, Szeged, Hungary

Associate Editors

for Anaerobes in Human Infections:

R.M.C.P Domingues • H. Mikamo • V. O. Rotimi

for Angerobes in Animal Disease: T.G. Nagaraja • F.A Uzal

for Antimicrobial Susceptibility of Anaerobic Bacteria: D. Citron • J. Soki

for Case Reports: E.J.C. Goldstein • H. Pituch

for Anaerobes in the Microbiome: E. Allen-Vercoe • A.B. Onderdonk

for Clostridium Difficile: S. Johnson • P. Mastrantonio • M. Rupnik

for Clinical Microbiology: U.S. Justesen • B. Limbago • A. Schuetz

for Pathogenesis and Toxins: C. Coursodon Boyiddle • S. Patrick

for Molecular Biology and Genetics: M.J. Gillespie • D. Lyras

Review Editor: D. Aronoff



Anaerobe is essential reading for those wishing to remain at the forefront of discoveries relating to life processes of strict anaerobes. The journal is multi-disciplinary, and provides a unique forum for researchers investigating infections caused by anaerobic bacteria in humans and animals. Anaerobe publishes original research articles, notes and case reports. Papers describing innovative methodologies, technologies, and applications are also of interest. Reviews are welcomed at the invitation of the editor.

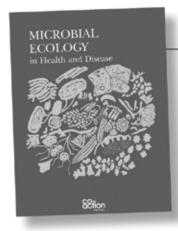
Benefit from:

- Rapid publication: quick reviewing and publication times
- Peer review: rigorous peer review on all published articles
- Flexible publication: open access options available

For the full aims & scope, or to submit your article online, visit: journals.elsevier.com/anaerobe

PUBLISH YOUR WORK OPEN ACCESS

FOR WIDE DISSEMINATION



MICROBIAL ECOLOGY IN HEALTH & DISEASE

Microbial Ecology in Health & Disease (MEHD) draws together research on eco-systems to increase our understanding of their role in health and disease. MEHD is the official journal of the Society for Microbial Ecology and Disease (SOMED) and is recognized by the Oral Microbiology and Immunology Group (OMIG) of the British Society for Dental Research (BSDR). All articles are deposited immediately upon publication with PubMed/PubMed Central.

www.MicrobEcolHealthDis.net

JOURNAL OF ORAL MICROBIOLOGY

As the first Open Access peer-reviewed journal in its field, Journal of Oral Microbiology (JOM) is becoming an influential source of knowledge on the aetiological agents behind oral infectious diseases. The journal covers research on pathogenesis, virulence, host-parasite interactions, and immunology of oral infections.

All articles are deposited immediately upon publication with PubMed/PubMed Central. First official Impact Factor to be awarded for 2015.





Join MEHD and JOM

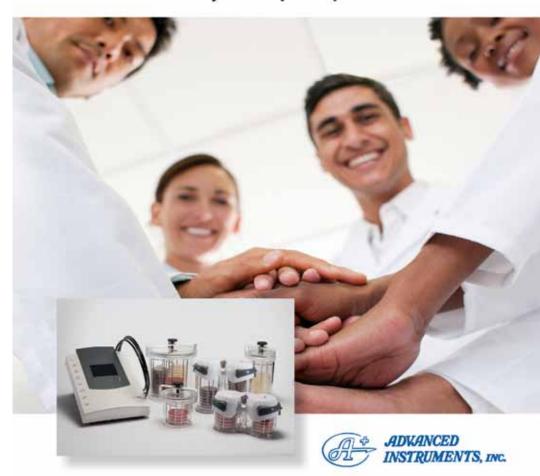


www.co-action.net



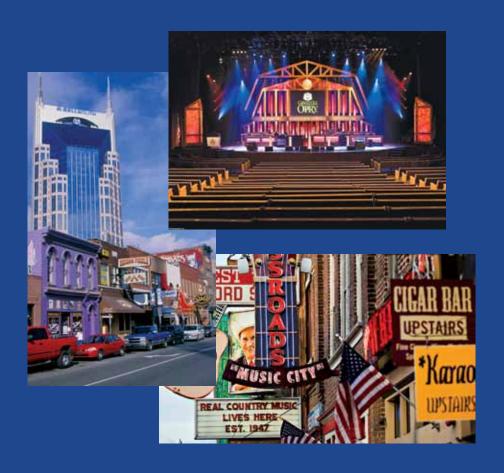


Create anaerobic environments that allow your people to thrive



Anoxomat from Advanced Instruments is the precise solution for everyone:

- > Lab managers appreciate the lower cost of ownership and smaller footprint.
- > Clinicians and researchers enjoy faster, more precise results.
- > Lab techs like the repeatability and the way it fits into their workflow. Plus, they love our new ergonomic jars that are easier to use, lighter to carry, and take up far less room.





ANAEROBE SOCIETY OF THE AMERICAS

PO Box 452058

Los Angeles, CA 90045

Phone: 310-216-9265 Fax: 310-216-9274 Web: www.anaerobe.org E-mail: asa@anaerobe.org

©2016 Anaerobe Society of the Americas