Program & Abstract Book

2014 Anaerobe

The 12th Biennial Congress of the
Anaerobe Society of the Americas

The 37th Congress of the
Society for Microbial Ecology and Disease

Westin River North Hotel
Chicago, Illinois USA
June 28-July 1, 2014

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Contents

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Welcome to Anaerobe *2014*, a joint meeting of the Anaerobe Society of the Americas (ASA) and the Society for Microbial Ecology and Diseases. 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 *2014* again illustrates the international interest in the field of anaerobic bacteriology. The 189 papers included in this Abstract Book represent the work of nearly 600 scientists from more than 30 countries.

At this Congress, the Keynote Address will be given by Dr. Martin J. Blaser of New York University. Dr. Blaser is investigating the human microbiome and its effect on various disease conditions. He has recently authored the book, *Missing Microbes: How the Overuse of Antibiotics is Fueling Our Modern Plagues*.

The Lifetime Achievement Award will be presented to Mike Cox of Anaerobe Systems. Mike has been on the forefront of promoting anaerobic bacteriology, as the developer of laboratory technology, educator of laboratory methodology, and application of anaerobic bacteria in energy production.

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 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.

Our hope is that Anaerobe *2014* serves to foster stimulating discussions, as well as cultivate personal relationships that continue to invigorate the entire field beyond the timeframe of this Congress.

Dale N. Gerding, M.D.
ASA President

Andrew B. Onderdonk
SOMED President

About the Anaerobe Society

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

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

About SOMED

Founded in 1988, the Society for Microbial Ecology and Disease is a non-profit scientific society, with the mission to promote scientific knowledge and encourage research and technology in the field of microbial ecology and its relation to diseases. The Society conducts annual Congresses and publishes the journal *Microbial Ecology in Health and Disease*. The Society encourages the participation of an interdisciplinary group of scientists in the study of the role of the microbiome in health and disease.

Further information and membership forms are available at the SOMED website: *www.somed.nu/*

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

Support for this activity from commercial interests include:

Platinum Patrons

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Bronze Patrons

- Actelion Pharmaceuticals
- Alere
- Anaerobe Journal / Elsevier
- BDC Food
- TechLab

Exhibitors

- Advanced Instruments
- Bruker Daltonic
- Cepheid
- Coy Laboratory Products
- Hardy Diagnostics
- Key Scientific Products
- List Biological Laboratories
- Microbiology International
- Shel Lab

Keynote Speaker

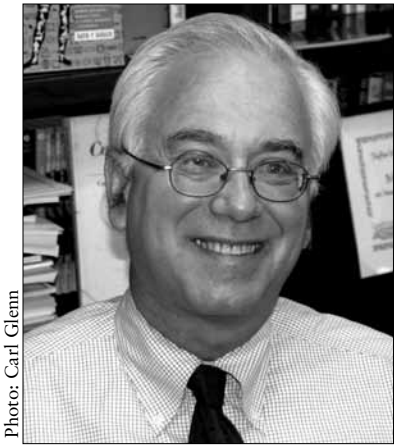


Photo: Carl Glenn

Martin J. Blaser, M.D.

Martin J. Blaser, M.D., is the George and Muriel Singer Professor of Medicine, Professor of Microbiology, and Director of the Human Microbiome Program at the NYU School of Medicine. He served as Chair of the Department of Medicine at NYU (2000-2012).

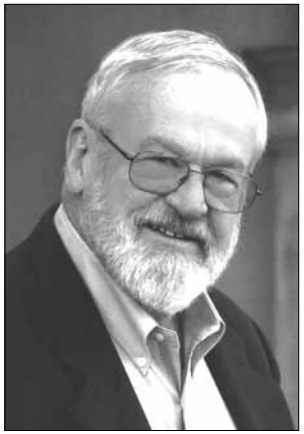
Dr. Blaser did his undergraduate work at the University of Pennsylvania in 1969, graduated from the New York University School of Medicine in 1973, and did his post-graduate training at the University of Colorado School of Medicine (1973-1979). He then was an Epidemic Intelligence Service Officer at the Centers for Disease Control and Prevention (1979-1981).

A physician and microbiologist, Dr. Blaser is interested in understanding the relationships that we have with our persistently colonizing bacteria. His work over the past 30 years focused on human pathogens, including Campylobacter species and *Helicobacter pylori*, which also are model systems for understanding interactions of residential bacteria with their human hosts. Over the last decade, he has been actively studying the relationship of the human microbiome to health and such important diseases as asthma, obesity, diabetes, and allergies.

Over the course of his career, Dr. Blaser has served as the advisor for a large number of students, post-doctoral fellows, and junior faculty, and he has been actively involved in national scientific and professional organizations. He served as President of the Infectious Diseases Society of America, Chair of the Board of Scientific Counselors of the National Cancer Institute, Chair of the Advisory Board for Clinical Research of the National Institutes of Health, and Founder of the Foundation for Bacteriology and the Virtual Museum of Bacteria. He currently serves on the Scientific Advisory Board of the Doris Duke Charitable Foundation.

He was elected to the Institute of Medicine and the American Academy for Arts and Sciences. He holds 24 U.S. patents relating to his research and has authored over 500 original articles. Most recently, he wrote *Missing Microbes: How the Overuse of Antibiotics Is Fueling Our Modern Plagues*, explaining the harmful effects of overusing antibiotics to the general public.

Lifetime Achievement Award



Marion (Mike) E. Cox, B.S., M.T.

Mike Cox has been a major advocate for the field of anaerobic bacteriology. As C.E.O. of Anaerobe Systems in Morgan Hill, CA, Mike has pioneered technology for the lab, while traveling across the country and around the world educating laboratory scientists on the proper methods of anaerobic identification and susceptibility.

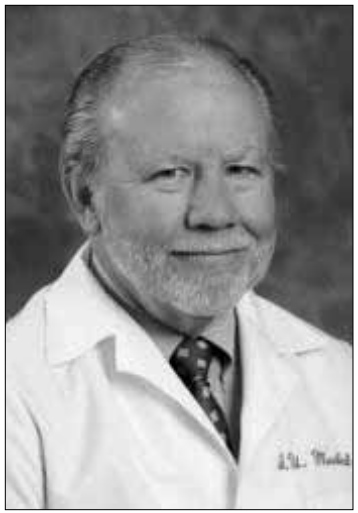
Mike became interested in microbiology while in the Army stationed at Fort Ord in Monterey, CA, and then completed his Bachelor degree in Biology at the University of Houston in 1973. He went on to become a microbiologist at Stanford University (1973-75) and then as Director of Research at International Shellfish Enterprises, he investigated causes of massive mortalities of oysters in this hatchery (1975-77).

While working in the clinical microbiology lab, he used VPI roll tube technique and candle jars for his anaerobe workups and wanted to create a better method for culturing anaerobic bacteria. In 1978, Mike founded Anaerobe Systems to develop new technology and products in the field of Microbiology. His patents include the gloveless anaerobic chamber, preparation of anaerobic media, and anaerobic production of hydrogen and other chemical products. His Pre-Reduced Anaerobically Sterilized (PRAS) plated and tubed culture media are packaged and pre-reduced in oxygen-free foil packages, requiring no preconditioning, saving many hours of lab work and anaerobic gas.

Over his 35 year career as a microbiologist, he has conducted hundreds of workshops on anaerobic methods, including the Pre-Congress Workshops offered with every Anaerobe Society Congress. He has also encouraged anaerobic studies at such schools as Santa Clara University, offered internships, and assisted students to attend the Anaerobe Congresses.

Mike serves as a Counsellor of the Anaerobe Society of the Americas, as well as a member of industry subcommittees on Antimicrobial Susceptibility Testing and Abbreviated Bacterial Identification. And in recent years, he has been involved in pioneering the use of microbes to create clean energy from food and agricultural waste.

In addition, Mike has been very active within his home community. He annually underwrites the Morgan Hill Youth Leadership, as well as being a generous contributor to such local organizations as the Morgan Hill Community Foundation, Friends of the Morgan Hill Library, AAUW, Rotary Club of Morgan Hill, the Morgan Hill Historical Society, and many science and education programs. In appreciation for his contribution to his community, he was bestowed with the 2013 Leadership Excellence Award by Leadership Morgan Hill.



Stephen Allen, M.D. (1943-2012)
Dr. Allen was the James Warren Smith Professor of Pathology and Laboratory Medicine and Director of Clinical Microbiology Laboratories at Indiana University (IU) Health and VA Medical Center in Indianapolis. He was also President of The Anaerobe Society (2003-2004), overseeing *Anaerobe 2004* in Annapolis, MD.

He earned his Undergraduate and Masters degree in Microbiology from IU Bloomington and MD degree from the IU School of Medicine in 1970. He did his Pathology Residency at Vanderbilt University and served as Medical Officer with the United States Public Health Service at the Centers for Disease Control and Prevention in Atlanta, GA (1974-1977).

Dr. Allen's appointment in the IU Pathology Department began in 1977, where he was responsible for the administration of Clinical Microbiology Laboratories at IU Health, Wishard, and the Veterans Administration Hospitals. He also served as Director of the Disease Control Laboratories with the Indiana State Board of Health (1994-2004).

He belonged to many Medical and Pathology Societies and received numerous awards and recognitions including membership in the Alliance of Distinguished and Titled Professors.

He served as a Trustee for the American Board of Pathology (1995-2006) and President of that Board (2003). Through authorship and co-authorship of 30 text books and several hundred publications, Dr. Allen became respected internationally as a researcher and educator of his field of anaerobic microbiology.

Steve was 69.

Abigail A. Salyers, Ph.D. (1942-2013)
Dr. Salyers, was known worldwide as a research scientist, author, and professor at the University of Illinois at Urbana-Champaign and was the Keynote Speaker at *Anaerobe 2004*.

Abigail had an undergraduate degree in mathematics and a Ph.D. in nuclear physics from George Washington University, Washington, D.C. After four years of teaching, research, and tenure at St. Mary's College in Maryland, she switched fields by taking courses in biochemistry and microbiology and secured a second post-doctorate position in biochemistry and microbiology from Virginia Polytechnical Institute. She studied, taught and did research at VPI (1973-1978).

She came to University of Illinois in 1978 and became the first female tenured professor in microbiology in 1983 and a full professor in 1988. While at Illinois, Abigail was named a University Scholar, Faculty Member of the Year in the College of Medicine, a member of the Center for Advanced Study, and an Affiliate in the Institute for Genomic Biology.

She received the Pasteur Award for Research and Teaching, the All-Campus Award for Excellence in Teaching in the UI Medical School, and the Golden Apple Award for Medical School Teaching three times. She was named the G. William Arends Professor in Molecular and Cellular Biology from 2004 until she retired in 2012.

She authored five books—including *Bacterial Pathogenesis: A Molecular Approach* (1994), *Bacterial Resistance to Antimicrobials* (2002), and *Revenge of the Microbes* (2005)—and 200 peer-reviewed research articles, reviews, and chapters in books. Her papers were cited widely (over 600 citations).

Abigail was President of the American Society for Microbiology (2001-2002). Her research was supported by the Department of Energy and the National Institutes of Health. In recognition of her standing in the scientific community, she served several terms as a member of National Institutes of Health panels that reviewed research grants. She was awarded an honorary doctorate from ETH University in Zurich, Switzerland (2001).

Abigail was 70.

In Memoriam

Felicja Meisel-Mikolajczyk, M.D., Ph.D.
(1927-2012)
Dr. Meisel-Mikolajczyk was Professor of Medical Microbiology at the Medical University of Warsaw in Poland. She was a long-time member of the Anaerobe Society and a regular poster presenter at the Anaerobe Congresses.

She came from a family of doctors. Her parents worked at the National Institute of Hygiene, but after the 1939 German invasion of Poland, they were rescued from the Livov Jewish Ghetto by Professor Rudolf Weigl to work at his Institute for the Study of Typhus and Viruses on developing the anti-typhus vaccine. However, they were arrested in 1943 and deported to the Auschwitz concentration camp. Felicja, on the other hand, hid with people of "good will"—including Catholic nuns running an orphanage—through the war years, moving to and from 18 different places. At the conclusion of the war, she was reunited with her parents.

In 1952, she graduated from the Medical Academy of Medicine in Warsaw (now called the Medical University of Warsaw) and continued to work in its Department of Medical Microbiology. She became a Full Professor in 1985 and headed the department (1992-1997). She guided generations of microbiologists. Even after her retirement in 1997, she still came to work every day to supervise studies and write articles for publication.

Prof. Meisel-Mikolajczyk's scientific work mainly pertained to anaerobes. Her initial research focused on *Clostridium perfringens* (the antigenic construction of vegetative forms and spores of this bacteria) and, later, non-sporulating anaerobes. In the 1980s, her focus was *Bacteroides fragilis* and was the first researcher in Poland to investigate *Clostridium difficile*. She collaborated with researchers around the world, producing more than 180 publications.

She was a member of a number of scientific societies, both domestic and international. Besides the numerous honors bestowed upon her by the Medical University, she received the national awards of the Golden Cross of Merit and the Order of Polonia Restituta Polish, both the highest awards for a Polish citizen.

Felicja was 85.

Accreditation/ Goals & Objectives

Anaerobe *2014*—the 12th biennial Congress of the Anaerobe Society of the Americas and the 37th Congress of the Society for Microbial Ecology and Disease—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 19.0 continuing education contact hours upon completion of the program and 7.0 continuing education contact hours upon completion of the 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 obtained by completing the request form in your Delegate Packet and returning it to the Registration Table with completed evaluation forms for sessions attended. 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 xvi-xvii.

Evaluation Forms

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

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Disclosure Information

Anaerobe *2014*

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

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

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

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

Participant Disclosure

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

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Christine M. Bassis, Ph.D.
Martin J. Blaser, M.D.
Alojz Bomba, D.Sc.
Laurent Bouillaut, Ph.D.
Georg Conrads, Ph.D.
Daniel B. DiGiulio, M.D.
Adam Driks, Ph.D.
Adrianne N. Edwards, Ph.D.
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Dennis L. Stevens, M.D., Ph.D.
Kerin L. Tyrell
Meera Unnikrishnan, Ph.D.
Vincent B. Young, M.D.

The following presenters have information to disclose as follows:

Ernesto Abel-Santos, Ph.D.	SporDiff (O)
Johan S. Bakken, M.D., Ph.D	None Will discuss fecal microbiota transplantation
Ellen Jo Baron, Ph.D.	Cepheid (E, O)
Eugenia Bezirtzoglou, M.D., Ph.D.	Wyeth (R)
Nadiya V. Boyko, D.Sc.	None Will discuss future products
Paul Carlson Jr., Ph.D.	Coy Lab Products (G)
Diane M. Citron	Toltec (G)
Laurie M. Cox, Ph.D.	Anaerobe Systems (O)
Mike M. Cox	Anaerobe Systems (O)

Kevin Garey, Pharm.D.	Cubist (G), Merck (G), Summit (G) Will address future treatment strategies of *C. difficile*
Dale Gerding, M.D.	Actelion (C), Gojo (G), Merck (C), Rebiotix (C), Sanofi-Pasteur (C), Summit (C), Viropharma/Shire License (C) Will discuss non-toxigenic *C. difficile* for CDI prevention
Ellie J.C. Goldstein, M.D	Bio-K+ (C, G, S), Cerexa (G), Cubist (C, G, S), Forest (C, G, S), Merck (C, G, S), Novartis (C, G), Rempex (C, G) Will discuss antimicrobials in the pipeline
Yiping Han, Ph.D.	None Will discuss FadA as a diagnostic marker and a synthetic peptide to prevent *F. nucleatum* colonization
David W. Hecht, M.D., M.B.A.	bioMérieux (S)
Sharon Hillier, M.D.	Cepheid (G), Merck (C)
Curtis Huttenhower, Ph.D.	Danone Research (G), Seres Health (C, O)
Stuart Johnson, M.D.	Bio-K+ (C, S), Summit (C)
Purnima S. Kumar, D.D.S, Ph.D.	Phillips Oral Healthcare (G)
Pierre-Jean Maziade, M.D.	Bio-K+ (C, S)
Grigoria Mitropoulou, PH.D.	Wyeth (R)
Patricia J. Freda Pietrobon, Ph.D.	Sanofi Pasteur (E, O, S) Will discuss *C. difficile* vaccine
Dave H. Pincus	bioMérieux (E)
Glenn S. Tillotson, Ph.D.	Roche (C), Shionogi (C), Summit (C)
Gayatri Vedantam, Ph.D	Stellar Biopharmaceuticals (C, G)
Mark H. Wilcox, M.D.	Actelion (C, G), Astellas (C, G), Astra-Zeneca (C), Bayer (C), BioMérieux (G), Cubist (C, G), Durata (C), Johnson & Johnson (C), Merck (C), Nabriva (C), Novacta (C), Novartis (C), Optimer (C), Pfizer (C, G, S), Sanofi-Pasteur (C), Summitt (G), The Medicines Co. (C, G), VH Squared (C), Viropharma (C)
Alan J. Wolfe, Ph.D.	Astellas (G)

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

Saturday, June 28

730 **WORKSHOP & CONGRESS REGISTRATION OPENS**

830-1630 **ANAEROBE IDENTIFICATION WORKSHOP**

Sunday, June 29

700 **REGISTRATION / BREAKFAST / EXHIBITS**

800-810 **WELCOME REMARKS**

Dale N. Gerding, M.D., President, ASA
Andrew Onderdonk, Ph.D., President, SOMED

810-915 **SESSION I: ECOLOGY OF THE MICROBIOTA**

Moderator: *Wendy Garrett, M.D., Ph.D.*

SI-1 GI Microbiome and Immune Modulation
Richard S. Blumberg, M.D.

SI-2 Relating the Metatranscriptome and Metagenome of the Human Gut
Curtis Huttenhower, Ph.D.

SI-3 Functional Characterisation of a Unique Homologue of Ubiquitin Produced by the Commensal Gastro-Intestinal Bacterium *Bacteroides fragilis*
Maria T. Kowal, Ph.D.

915-930 **BREAK / EXHIBITS**

930-1030 **SESSION II: KEYNOTE ADDRESS**

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

SII-1 Our Disappearing Microbiota?
Martin J. Blaser, M.D.

1030-1040 **BREAK / EXHIBITS**

1040-1200 **SESSION III: MICROBIOTA: REACHING BEYOND THE GUT**

Moderators: *Cynthia L. Sears, M.D.*
Bennett Lorber, M.D.

SIII-1 Microbiota and Cancer Therapy
Romina S. Goldszmid, Ph.D.

SIII-2 What's Buggin You?: The Role of the Gut Microbiome in Central Nervous System Disease
Lloyd H. Kasper, M.D.

SIII-3 The Role of Early-Life Microbiota in Shaping Adult Body Composition
Laurie M. Cox, Ph.D.

Sunday, June 29

1200-1300 **LUNCH / EXHIBITS / COMMITTEE MEETINGS STUDENT COMPETITION PRESENTATIONS**

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

1400-1540 **SESSION IV: BENEFICIAL MICROBIOME MEMBERS**

Moderator: *Eugenia Bezirtzoglou, M.D., Ph.D.*

SIV-1 Probiotics—Rules and Problems
Tore Midtvedt, M.D., Ph.D.

SIV-2 Probiotics, Immunobiotics, and Diet in Predictive, Preventive, and Personalized Medicine
Nadiya V. Boyko, D.Sc.

SIV-3 Biodiversity of Intestinal Lactic Acid Bacteria and Health
Marika Mikelsaar, Ph.D.

SIV-4 Bifidobacteria and Rheumatism
Eugenia Bezirtzoglou, M.D., Ph.D.
Marie-Bénédicte Romond Ph.D.

SIV-5 Probiotics in Prevention of Chronic Diseases
Alojz Bomba, D.Sc.

SIV-6 *In vitro* Investigation of *Brachyspira pilosicoli*—Host Metabolic Interactions
Caroline I. LeRoy

1540-1545 **BREAK / EXHIBITS**

1545-1715 **SESSION V: ANAEROBES IN THE ORAL CAVITY**

Moderator: *Purima Kumar, D.D.S., Ph.D.*

SV-1 *Fusobacterium nucleatum*: A Commensal-Turned Pathogen
Yiping Han, Ph.D.

SV-2 Oral Bacteria and Systemic Disease: *P. gingivalis* Can Cause What?!
Richard J. Lamont, Ph.D.

SV-3 Oral Anaerobes in Health and Disease: 2014 Highlights
Georg Conrads, Ph.D.

SV-4 Highly Diverse Root Canal Microbiota in Chronic Apical Periodontitis According to Illumina Sequencing
Reet Mändar, M.D. Ph.D.

1715-1745 **ANAEROBE SOCIETY MEMBERSHIP MEETING**

1745-1900 **WINE & CHEESE RECEPTION**

Monday, June 30

700 **REGISTRATION / BREAKFAST / EXHIBITS**

730-830 **SATELLITE SYMPOSIUM I**

A New Look at Anaerobes in the Clinical Laboratory

SATI-1 MALDI-TOF MS for the Identification of Anaerobes
Dave H. Pincus

SATI-1 The Importance of Susceptibility Testing of Anaerobes: When, How, Why
David W. Hecht, M.D., M.B.A.

845-1025 **SESSION VI: BIOFILMS IN ANAEROBIC INFECTIONS**

Moderator: *David N. Fredricks, M.D.*

SVI-1 Microbial Biofilms and Colon Cancer
Cynthia L. Sears, M.D.

SVI-2 *Gardnerella vaginalis* Biofilms in Bacterial Vaginosis
Adam J. Ratner, M.D., M.P.H.

SVI-3 *Clostridium difficile* Biofilms (*in vitro*)
Adam Driks, Ph.D.

SVI-4 The Microbiome in Oral Health and Disease
Purnima S. Kumar, D.D.S, Ph.D.

1025-1035 **BREAK / EXHIBITS**

1035-1150 **SESSION VII: DIAGNOSTIC AND LABORATORY TECHNIQUES**

Moderator: *Diane M. Citron*

SVII-1 Oscillibacter Who? What Shall We Do with These New Names?
Diane M. Citron

SVII-2 European Experience with Maldi-TOF MS in the Field of Anaerobes
Elisabeth Nagy, M.D., Ph.D., D.Sc.

SVII-3 Animal Models for *Clostridium difficile* Infection
Vincent B. Young, M.D.

1150-1250 **LUNCH / EXHIBITS**

1250-1350 **POSTER SESSION II / EXHIBITS**

1350-1440 **SESSION VIII: THE CARE AND FEEDING OF OUR INTESTINAL MICROBIOME**

Moderator: *Andrew Onderdonk, Ph.D.*

SVIII-1 Functional Dairy Foods and Human Intestinal Microflora
Eugenia Bezirtzoglou, M.D., Ph.D.

SVIII-2 New Antimicrobial Strategy Based on Inhibition of Pathogen's Cell-to-Cell Signaling and Consecutive Virulence Features Expression by Probiotic Signal Molecules
Veronica Lazar, Ph.D.

Monday, June 30

1440-1600 **SESSION IX: *CLOSTRIDIUM SPP:* HEALTH AND DISEASE**

Moderator: *Carl Erik Nord, M.D.*

SIX-1 The Opportunistic Pathogen *Clostridium speticum*
David M. Aronoff, M.D.

SIX-2 Life-Threatening Toxin Mediated Clostridial Infections
Dennis L. Stevens, M.D., Ph.D.

SIX-3 Spores of *Clostridium* Engineered for Clinical Efficacy and Safety Cause Regression and Cure of Tumors *in vivo*
Nigel P. Minton, Ph.D.

SIX-4 *Clostridium sordellii* Infections: Insights into the Pathogenesis of Disease
Mike J. Aldape, Ph.D.

SIX-5 Identification of the Host Receptor for *Clostridium perfringens* TpeL Toxin Indicates a Two-Receptor Model of Clostridial Glycosylating Toxins
Panagiotis Papatheodorou, Ph.D.

1600-1610 **BREAK / EXHIBITS**

1610-1710 **SESSION X: WHOLE GENOME SEQUENCING**

Moderator: *Ellen Jo Baron, Ph.D.*

SX-1 Genomic Investigations of the Maternal Microbiome During Pregnancy and its Association with Preterm Birth
Daniel B. DiGiulio, M.D.

SX-2 Urine is Not Sterile: New Tools Lead to New Hypotheses
Alan J. Wolfe, Ph.D.

1710-1800 **SESSION XI: ANTIMICROBIALS AND RESISTANCE**

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

SXI-1 The Drug Pipeline for Anaerobic Infections
Ellie J.C. Goldstein, M.D.

SXI-2 Antimicrobial Susceptibility Testing in Europe
Carl Erik Nord, M.D.

SXI-3 Emergence and Evolution of an International Clone of Multiresistant *Bacteroides fragilis* Isolates
József Sóki, Ph.D.

SXI-4 Evaluation of the Antimicrobial Susceptibility Profiling of Tigecycline and Other Antibiotics against Clinical Isolates
Grigoria Mitropoulou, Ph.D.

1815 **CONGRESS BANQUET RECEPTION / FULTON'S ON THE RIVER**

1915 **CONGRESS BANQUET & AWARDS**

FINEGOLD AWARD • YOUNG INVESTIGATORS AWARDS

LIFETIME ACHIEVEMENT AWARD: Mike Cox, Anaerobe Systems

Tuesday, July 1

700

REGISTRATION / BREAKFAST / EXHIBITS

730-830

SATELLITE SYMPOSIUM II

Primary Prevention of Clostridium difficile Infection with Specific Probiotics

SATII-1 Meta-analysis of Probiotic Primary CDI Prevention Studies
Stuart Johnson, M.D.

SATII-2 Impact of Universal Probiotic Administration to Antibiotic Recipients in a Community Hospital
Ellie J.C. Goldstein, M.D.

845-1000

SESSION XII: VAGINAL MICROBIOME

Moderator: *Raina N. Fichorova, M.D, Ph.D.*

SXII-1 The Vaginal Microbiome: What's New Since 2012?
Jeanne M. Marrazzo, M.D.

SXII-2 Which Members of the Vaginal Microbiome are Associated with Amniotic Fluid Infections and PID?
Sharon Hillier, M.D.

SXII-3 The Differential Roles of Vaginal Bacteria in Tuning Mucosal Immunity
Raina N. Fichorova, M.D, Ph.D.

SXII-4 Effect of Intrauterine Contraception on Vaginal Microbiota
Christine M. Bassis, Ph.D.

1000-1015 BREAK / EXHIBITS

1015-1145

SESSION XIII: *CLOSTRIDIUM DIFFICILE* EPIDEMIOLOGY & PREVENTION

Moderator: *Stuart Johnson, M.D.*

SXIII-1 Update on the Epidemiology of *Clostridium difficile* Infections from the CDC
L. Clifford McDonald, M.D.

SXIII-2 New Insights into *Clostridium difficile* Transmission in the Hospital Based on Whole Genome Sequencing
Mark H. Wilcox, M.D.

SXIII-3 Development and Progression of a Candidate *Clostridium difficile* Vaccine for the Prevention of Symptomatic CDI
Patricia J. Freda Pietrobon, Ph.D.

SXIII-4 Laboratory-Based Surveillance of *Clostridium difficile* Strains Circulating in Australian Healthcare
Thomas V. Riley, Ph.D.

SXIII-5 Does Binary Toxin Contribute to *Clostridium difficile* Infection?
Sarah A. Kuehne, Ph.D.

1145-1245 LUNCH / EXHIBITS

1245-1345 POSTER SESSION III / EXHIBITS

Tuesday, July 1

1345-1500

SESSION XIV: *CLOSTRIDIUM DIFFICILE* TREATMENT & IMMUNITY

Moderator: *David M. Aronoff, M.D.*

SXIV-1 Optimizing Existing Therapies for *Clostridium difficile* Infections
Stuart Johnson, M.D.

SXIV-2 Emerging Drugs and Vaccines against *Clostridium difficile:* A New Strategy for the Prevention
Kevin Garey, Pharm.D.

SXIV-3 A New Strategy for the Prevention of *Clostridium difficile* Infections
Ernesto Abel-Santos, Ph.D.

SXIV-4 Global Analysis of the Role of Inflammation in *Clostridium difficile* Colonization and Disease
Gary B. Huffnagle, Ph.D.

1500-1510 BREAK / EXHIBITS

1510-1610

SESSION XV: BACTERIAL THERAPY

Moderator: *Vincent B. Young, M.D., Ph.D.*

SXV-1 Non-Toxigenic *Clostridium difficile* (NTCD) for Prevention of Recurrent *C. difficile* Infection (CDI)
Dale N. Gerding, M.D.

SXV-2 Treatment Approaches for Resolving Recurrent *Clostridium difficile* Infections
Johan S. Bakken, M.D., Ph.D.

1610-1740

SESSION XVI: NEW INSIGHTS INTO *CLOSTRIDIUM DIFFICILE* PATHOGENESIS

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

SXVI-1 In-Depth Proteomic Analysis of the Toxigenic and Non-Toxigenic *Clostridium difficile* Secretome
Hercules Moura, Ph.D.

SXVI-2 Different Ways to Die: Epidemic-Associated *Clostridium difficile* Remodels its Cell-Surface, and Manipulates the Host Innate Immune System to Cause Severe Disease
Gayatri Vedantam, Ph.D.

SXVI-3 Intersection of Metabolism and Pathogenesis in *Clostridium difficile*
Laurent Bouillaut, Ph.D.

SXVI-4 Conserved Oligopeptide Permeases Modulate Sporulation Initiation in *Clostridium difficile*
Adrianne N. Edwards, Ph.D.

SXVI-5 Defining the Early Stages of *Clostridium difficile* Spore Germination
Joseph A. Sorg, Ph.D.

SXVI-6 Complex Regulation of *Clostridium difficile* Biofilms
Meera Unnikrishnan, Ph.D.

SXVI-7 Mechanisms of Iron Acquisition in *Clostridium difficile*
Paul Carlson Jr., Ph.D.

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.

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
- SAT—Satellite Symposium

*Indicates Presenting Author

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

810 *SESSION I:* ECOLOGY OF THE MICROBIOTA

GI MICROBIOME AND IMMUNE MODULATION

Blumberg, R.S.*
Brigham and Women's Hospital, Boston, MA, USA

RELATING THE METATRANSCRIPTOME AND METAGENOME OF THE HUMAN GUT

Huttenhower, C.*
Harvard University, Cambridge, MA, USA

Although the composition of the human microbiome is now well-studied, the microbiota's >8 million genes and their regulation remain largely uncharacterized. This knowledge gap is in part because of the difficulty of acquiring large numbers of samples amenable to functional studies of the microbiota. We conducted what is, to our knowledge, one of the first human microbiome studies in a well-phenotyped prospective cohort incorporating taxonomic, metagenomic, and metatranscriptomic profiling at multiple body sites using self-collected samples. Stool and saliva were provided by eight healthy subjects, with the former preserved by three different methods (freezing, ethanol, and RNAlater) to validate self-collection. Within-subject microbial species, gene, and transcript abundances were highly concordant across sampling methods, with only a small fraction of transcripts (<5%) displaying between-method variation. Next, we investigated relationships between the oral and gut microbial communities, identifying a subset of abundant oral microbes that routinely survive transit to the gut, but with minimal transcriptional activity there. Finally, systematic comparison of the gut metagenome and metatranscriptome revealed that a substantial fraction (41%) of microbial transcripts were not differentially regulated relative to their genomic abundances. Of the remainder, consistently underexpressed pathways included sporulation and amino acid biosynthesis, whereas up-regulated pathways included ribosome biogenesis and methanogenesis. Across subjects, metatranscriptional profiles were significantly more individualized than DNA-level functional profiles, but less variable than microbial composition, indicative of subject-specific whole-community regulation. The results thus detail relationships between community genomic potential and gene expression in the gut, and establish the feasibility of metatranscriptomic investigations in subject-collected and shipped samples.

FUNCTIONAL CHARACTERISATION OF A UNIQUE HOMOLOGUE OF UBIQUITIN PRODUCED BY THE COMMENSAL GASTRO-INTESTINAL BACTERIUM *BACTEROIDES FRAGILIS*

Kowal, M.T.;*[1] Patrick, S.;[2] Blakely, G.W.[1]
[1]Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK
[2]Centre for Infection and Immunity, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, UK

Bacteroides fragilis is a Gram negative, anaerobic commensal of the human gastro-intestinal tract known to have important roles in development of the host immune system and intestinal permeability. *B. fragilis*, however, is also an opportunistic pathogen commonly isolated from bacteraemia and anaerobic abscesses. Recently, *B. fragilis* was shown to encode and express a unique protein, BfUbb, which shares 63% sequence identity with human ubiquitin. BfUbb is exported to the periplasm and packaged into outer membrane vesicles which might transport proteins through the mucosa and deliver them to host cells. BfUbb has evolved two key differences to eukaryotic ubiquitin: the addition of an N-terminal periplasmic signal sequence and the substitution of the C-terminal glycine residues with a cysteine residue. Possibly due to these differences, BfUbb is able to terminate polyubiquitination *in vitro* by binding to the human E1 activating enzyme.

Our work utilizes different approaches to examine the effects of BfUbb on host cells. We use microscopy to examine BfUbb localization and morphological changes mediated by BfUbb when expressed in different cell types. Biochemical studies demonstrate that BfUbb is able to bind to multiple E2 proteins of the ubiquitination pathway *in vitro*, independently of both ATP and the E1 activating enzyme. In addition, co-purification experiments allow identification of other potential targets *in vivo*. These data suggest that BfUbb targets specific proteins in the eukaryotic ubiquitination pathway. Such activity has the potential to interfere with a wide range of host cell processes, including modulation of the inflammatory response.

SII-1

OUR DISAPPEARING MICROBIOTA?

Blaser, M.J.*
New York University Langone Medical Center, New York, NY, USA

Normal flora are normal; commensals are not supposed to be lost from generation to generation. Yet *Helicobacter pylori*, the ancient, highly host-interactive, and cosmopolitan bacteria that have dominated the gastric niche have been progressively disappearing from humans over the last century. Not surprisingly, this change in human microecology has had health consequences, and also not surprisingly, these are both good and bad. If one member of the normal flora is disappearing, might there be others, and might there be other health consequences?

For 70 years, farmers have been giving low doses of antibiotics to their livestock to promote their growth. This practice is effective, and the earlier in life it is started, the more effective. We postulated that a change in the early life microbiota is affecting development. Studies in mouse models provides evidence for this view, and importantly, changes in the microbiota affect gene transcription in the host.

We postulate that these are representative of changes in the early life human microbiota that are fueling the metabolic, allergic, and auto-immune diseases becoming epidemic in the developed world.

1040 ***SESSION III:*** **MICROBIOTA: REACHING BEYOND THE GUT**

MICROBIOTA AND CANCER THERAPY

Goldszmid, R.S.*
Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, NIH, Frederick, MD, USA

Mammals live in partnership with a rich commensal microbiota on the body epithelial surfaces. This partnership is critical for tissue formation, metabolism and the development and function of the innate and adaptive resistance. The gut microbiota plays a role not only in local tissue formation and the development of mucosal immunity, but has also systemic effects regulating inflammation, immunity, metabolic, cardiovascular and neurological functions. The microbiota is closely linked to cancer development, both locally (e.g. colorectal carcinoma) and at distant sites (mammary carcinoma, hepatocellular carcinoma, lymphoma), and it is also involved in the establishment of metabolic pathologies favoring cancer (e.g. obesity). The local effects of the microbiota can be explained by the interaction of bacteria or their products with innate receptors or other sensors on epithelial cells or associated hematopoietic cells. However, the cellular and molecular mechanisms by which the gut commensal microbiota regulates the threshold of activation of systemic innate and adaptive immunity are only beginning to be characterized.

Although the role of inflammation in cancer is well documented, whether changes in microbiota composition affect the inflammation in the sterile tumor microenvironment remains unclear. We have recently shown that microbiota perturbation impairs the response of subcutaneous cancers to CpG oligonucleotide-immunotherapy or platinum chemotherapy, and in both cases innate myeloid cells are responsible for the impaired response albeit through distinct mechanisms. Thus, optimal response to cancer immunotherapy and chemotherapy requires an intact commensal microbiota that acts distantly by modulating myeloid-derived cell function in the tumor microenvironment.

WHAT'S BUGGIN YOU?: THE ROLE OF THE GUT MICROBIOME IN CENTRAL NERVOUS SYSTEM DISEASE

Kasper, L.H.*
Geisel School of Medicine, Dartmouth College Hanover, NH, USA

Mammals live in a co-evolutionary association with the plethora of microorganisms that reside at a variety of tissue microenvironments. The microbiome represents the collective genomes of these co-existing microorganisms, which is shaped by host factors such as genetics and nutrients but in turn is able to influence host biology in health and disease. Niche-specific microbiome, prominently the gut microbiome, has the capacity to effect both local and distal sites within the host. The gut microbiome has played a crucial role in the bidirectional gut–brain axis that integrates the gut and central nervous system (CNS) activities, and thus the concept of microbiome–gut–brain axis is emerging. Studies are revealing how diverse forms of neuro-immune and neuro-psychiatric disorders are correlated with or modulated by variations of microbiome, microbiota-derived products and exogenous antibiotics and probiotics. The microbiome poises the peripheral immune homeostasis and predisposes host susceptibility to CNS auto-immune diseases such as multiple sclerosis. Neural, endocrine and metabolic mechanisms are also critical mediators of the microbiome–CNS signaling, which are more involved in neuro-psychiatric disorders such as autism, depression, anxiety, stress. Research on the role of microbiome in CNS disorders deepens our academic knowledge about host-microbiome commensalism in central regulation and in practicality, holds conceivable promise for developing novel prognostic and therapeutic avenues for CNS disorders.

SIII-3

THE ROLE OF EARLY-LIFE MICROBIOTA IN SHAPING ADULT BODY COMPOSITION

Cox, L.M.*
Laboratory of Martin Blaser, Department of Medicine, NYU Langone Medical Center, New York, NY, USA

Acquisition of the intestinal microbiota begins at birth and a stable microbial community develops from a succession of key organisms. Antibiotic disruption of the co-evolved microbiota during maturation can increase weight and adiposity. To investigate the role of timing, diet, and causation, C57BL/6J mice received low-dose penicillin (LDP) or not (control) in several murine models of growth promotion. We show that low-dose penicillin (LDP) delivered from birth enhanced metabolic alterations compared to delivered at weaning, and that there were additive effects in combination with high fat diet. LDP that was limited to early life was sufficient for sustained effects on body composition. After the cessation of antibiotics, the microbial communities recovered, yet metabolic phenotypes persisted, indicating that microbiota interactions in infancy may be critical determinants of long-term host effects. LDP reduced expression of ileal genes involved in immunity in early life, suggesting that microbe-immune interactions may mediate this effect. The growth promotion phenotype was transferrable to germ-free hosts by LDP-selected microbiota, demonstrating that the LDP-selected microbiota, not the antibiotics per se, alter host metabolism. These studies characterize important variables in early-life microbe-host metabolic interactions, and identify several taxa consistently linked with metabolic alterations.

1400 ***SESSION IV:*** **BENEFICIAL MICROBIOME MEMBERS**

PROBIOTICS—RULES AND PROBLEMS

Midtvedt, T.*
Department of Microbiology, Tumor and Cell Biology (MTC)
Karolinska Intsititute, Stockholm, Sweden

Microbiologically fermented food products have been used by man since ancient time but it was E. Metchnikoff—a Russian scientist—that in 1908 started to claim that fermented milk product could benefit human health. The term "probiotic" was brought into ecology more than 60 years ago, but was soon adopted by the food industry. Now there are close to 11,000 articles in PubMed, covering use of probiotics to man, animals and fish. For human consumption, most products are based on fermentation of milk by lactobacilli and/or bifidobacteria. Such products are sold worldwide and sales figures are steadily increasing. International companies, such as Danone, Nestle, and Yakult are present in many countries, leading to conformity in exposure to man. At our SOMED meeting in Yokohama in 2011, we discussed the rules regarding acceptance of health claims in Japan, Russia, USA and Europe. In Europe, EFSA (European Food Safety Agency) is currently going through claims regarding products on the European Market and a considerable discrepancy between claims and acceptance has been unmasked. Obviously, the producers have to improve their documentation of their products. At our SOMED meeting in Kosice last year, we discussed several important points, as genome and product characterization, long-term studies with regards to functional effects and safety etc. Now, we have started to learn that no probiotic product fits all. Based upon improved knowledge about epigenomic programming in early childhood and possible consequences for health later in life, there is an increasing awareness of giving probiotic products to infants. Another trend is to create probiotic products with very specific functions, and one such group, so-called "psychobiotics", will be commented upon and so will also the possibility of making genetically modified probiotic strains.

PROBIOTICS, IMMUNOBIOTICS, AND DIET IN PREDICTIVE, PREVENTIVE, AND PERSONALIZED MEDICINE

Boyko, N.V.;*[1,2] Spivak, N.Ya.[2,3]
[1]RD Centre of Molecular Microbiology and Mucosal Immunology, Uzhhorod National University, Uzhhorod, Ukraine
[2]"Cassovia Life Science", n.o., Kosice, Slovakia
[3]"DiaProph" Ltd., Kyiv, Ukraine

Cytokine response of immune cells vary depending on probiotic strains and the level of its production is crucial to determine the direction of Th1/Th2 reaction that should be taken into account when creating immunobiotics — preparations with precisely evaluated immunomodulatory activity. Prebiotics and probiotics are used as functional foods when incorporated into foods matrix. Diseases associated with diet (DAD) are initiated by low-grade inflammation and can be prevented by food since dietary intake alters specifically human gut microbiota and mucosal immune response.

The aim of our work is to provide the scientific evidence for the implementation of personalized diets for the protection of DAD in earlier stages. We had examined the content of prioritized traditional foods of plant origin[1] for the presence of biological active compounds and key microorganisms. Plants ingredients were tested for anti-/pro-microbial features against commensal, pathogenic and potentially pathogenic bacteria *in vitro*, *in silico* and *in vivo* models to be further defined as suitable components for novel functional foods. Edible plants of traditional foods induced a shift in expression of pro-/anti-inflammatory CD1a molecules on a surface of human DCs derived from peripheral blood monocytes. Commensal bacteria act on moDCs differentiation and activation by dose- and strain-specific manner, altering the expression of CD1a, NOD-2, RLH, PPARγ and RARα genes and cytokines production. Plant extracts and commensal microorganisms modulate the humoral and cellular mucosal immune response in GALT tissues on mice models differently regulating IgA secretion by peritoneal cavity B1/B2 cells in different compartments of small intestine, changing also the ratio of immune cells in mesenteric lymph nodes, Payer's patches, and spleen. Selected plants were able to affect endothelial function, lipid profile and insulin sensitivity of patients with metabolic and cardiovascular diseases in randomized controlled clinical trial study.

[1]Acknowledgements. Research funded by FP7 EU project BaSeFood, grant agreement n. 227118

BIODIVERSITY OF INTESTINAL LACTIC ACID BACTERIA AND HEALTH

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The biodiversity of intestinal microbiota has been associated with host health markers. In our previous studies the prevalence of *Lactobacillus* sp. (LB) species was related to geographical location, birth years and age. In the mid-1990s the Estonians compared to Swedish young children (1-2 y) expressed a higher biodiversity of intestinal *Lactobacillus* sp. composition (Mikelsaar *et al*., 2004). For instance, until the age of 6 months (born in 1996-1998) the rare colonization with *L. rhamnosus* (21% vs 62%, p=0.03) was found in Estonians compared to Swedes. This difference persisted still in the 2000s when the intestinal *L. rhamnosus* species was present only in 20% of Estonian adults (Stsepetova *et al*., 2011). It can be postulated that the individuality of *Lactobacillus* sp. composition is determined during early infancy. A study of Finnish children (born in 1995-2003) supported it by the substantial increase in the frequency of IgG antibody-reactive protein bands towards *L. rhamnosus* GG before the age of 12 months (Talja *et al*, 2014). Further, we assessed that the health indices such as weight, white blood cell count, blood glucose and ox-LDL levels were differentially bound to the large biodiversity of counts and particular intestinal LB species in both Estonian adults and elderly (Mikelsaar et al., 2010; Stsepetova *et al*., 2011). Early shifts in composition of lactic acid bacteria predicted the development of allergy: in two–years-old allergic children the counts of lactic acid bacteria (enterococci) were smaller at their newborn age (Björksten *et al*., 2003). At their age of 5 years, a less diverse composition of intestinal microbiota and a specifically higher prevalence *of Bifidobacterium adolescentis* compared to non-allergic children were shown (Stsepetova *et al*., 2007). Thus, the reduction of the biodiversity of intestinal lactic acid bacteria and a specific LB species composition are closely associated with impaired values of health biomarkers and disturbancies. In early infancy, the application of large variety of probiotic lactic acid bacteria may help to reduce the risk for metabolic and immune-mediated diseases.

BIFIDOBACTERIA AND RHEUMATISM

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Objective: Our aim was to investigate the potential of bifidobacterial macromolecules in alleviating rheumatoid arthritis and osteoarthritis and to determine the possible involvement of dendritic cells (DCs) and arthritic microbiota in Bifidobacterial Complex (BC) protective mechanism.

Methods & Results: A bifidobacterial complex produced during milk fermentation with *B.longum* was assayed for its anti-arthritic capacities in mouse arthritis and rat osteoarthritis models. BC at doses ranging between 15 and 100 µg/kg body weight reduces the arthritis index in mice. BC prevents cartilage degradation in rats at doses ranging between 10 and 20 µg/kg. BC was detected in bone marrow DCs from arthritic mice and in bone marrow and spleen DCs from healthy mice 5h and 15h post-oral ingestion. BC was then assayed in mice associated with the flora of an arthritic patient (RA) and spleen DCs gene expression was analyzed prior and after BC administration. DCs profile from mice with a healthy (HM) age matched individual was used as control. Bifidobacteria poorly colonized the RA gut. Splenic RA DCs were in lower numbers than HM DCs. RA and HM DCs differentially expressed 77 genes. Overexpression of 20 genes coding for catabolizing enzymes and down expression of *Fkbp5* evoked a decreased antigen presentation in RA DCs. Following 15 days of BC intake, bifidobacteria only slightly increased and the splenic DCs were still low. However, DCs genes profiling partially turned to a HM profile. Fourteen catabolizing genes and two genes involved in transport and exocytosis were down regulated and *Fkbp5* was up-regulated. It is suggested that antigen presentation by DCs was partially restored.

Conclusion: BC at anti-arthritic dosing abolished abnormal expression of several genes involved in DCs antigen presentation process. Competitive uptake of BC could prevent the pro-arthritic effects of bacteria from mouth and intestine. *Per os* administration of BC is a promising approach for alleviating rheumatisms.

PROBIOTICS IN PREVENTION OF CHRONIC DISEASES

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Chronic diseases are ranked among the most serious health problems in human medicine. Gut microbiota play a very important role in health maintenance and disease prevention. Dysbiosis is associated with a number of chronic diseases including obesity, metabolic syndrome, type 2 diabetes, inflammatory bowel diseases, atherosclerosis and cancer. The dysbiosis causes low-grade inflammation that is the key that links chronic diseases. Current knowledge shows that sophisticated modulation of gut microflora using probiotics and natural bioactive substances could effectively decrease the measure of health risks.

In our experiments, supplementation of the diet with probiotic strain *L. plantarum* reduced inflammatory processes occurring in the jejunal mucosa of rats exposed to N,N-dimethylhydrazine by inhibiting the production of pro-inflammatory cytokines and stimulating the regulatory cytokine IL-10. These findings suggest that *L. plantarum* can be used as a potent immunomodulator in prevention of chronic inflammatory diseases (Štofilová *et al*., 2013).

The results of our experiments using animal experimental models of atherosclerosis showed that probiotics beneficially influence gut microbiota composition and lipid metabolism by the reduction of plasma total cholesterol, LDL-cholesterol and triglycerides, depending on the characteristics of probiotic strain (Salaj *et al*., 2013).

Current knowledge suggests that chronic diseases shared a lot of common features including molecular pathways in pathogenesis. The results of animal experiments and clinical studies indicate that probiotics could influence the common molecular pathways of chronic disease pathogenesis. From that point of view, we suggested new definition of probiotics as follows: "Probiotics are live microorganisms which modulate the specific function of organism by activation of specific molecular pathways" (Bomba *et al*., 2012). Future research should be aimed to discover new effective combination of probiotics and natural bioactive substances that will be able to influence the pathogenesis of chronic diseases at different levels and molecular pathways.

Acknowledgements
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IN VITRO INVESTIGATION OF *BRACHYSPIRA PILOSICOLI*— HOST METABOLIC INTERACTIONS

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Avian intestinal spirochetosis (AIS) is a common European disease mainly caused by *Brachyspira pilosicoli*, a gram negative bacterium of the Spirochaetes phylum. During AIS, these opportunistic pathogens abnormally colonise the lower part of the bird's digestive tract, which is generally followed by typical symptoms such as diarrhoea, reduced growth rate and egg production. To prevent AIS, *Lactobacillus*-based probiotics appear to be a suitable solution but pleuromutilin antibiotics such as tiamulin remain the gold standard for treatment. This study aimed at understanding the metabolic impact of *B. pilosicoli* infection and both (antibiotic –tiamulin- and probiotic –*Lactobacillus salivarius*-) treatments on the host at a cellular and tissue level. Two independent infection and treatment assays were run: (1) on human derived HT29 cell lines cultivated in a 3D cell culture incubator and (2) in *in vitro* organ culture (IVOC) of chickens' ceca. To evaluate the impact of the probiotic treatment, protection, competition and displacement assays were performed and compared to three concentrations of tiamulin (0.250, 0.150 and 0.062 µg/ml). For both *in vitro* assays, media and tissues or cells were sampled after treatment and separated in order to analyse their metabolic profile by 1H-NMR-based metabonomics. Bacteriologic counts of the pathogen and the probiotic were also performed for 3D cell assays. Results showed a significant decrease in *B. pilosicoli* attachment on cells in protection and competition assays in 3D cell cultures. No living *B. pilosicoli* were found attached to the cells at the highest tiamulin dose. Furthermore, metabolic variations were observed in the media and tissues/cells, depending on treatment. These results confirm the metabolic impact of *B. pilosicoli* on the host during colonisation and infection in relation to a treatment. This study gives a preliminary overview of the host's metabolic response to AIS during infection and treatment, and encourages more research into the use of Lactobacillus-based probiotics as prophylactic treatments in industrial farms.

1545 *SESSION V:* ANAEROBES IN THE ORAL CAVITY

FUSOBACTERIUM NUCLEATUM: A COMMENSAL-TURNED PATHOGEN

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Fusobacterium nucleatum (*Fn*) is a Gram-negative oral commensal implicated in periodontal disease. It is highly prevalent in infections at extra-oral sites, associated with organ abscesses, pregnancy complications, and GI disorders. It was found as the predominant species associated with colorectal cancer (CRC). However, the causality and underlying mechanisms were not elucidated. Work from my laboratory demonstrates that *Fn* adheres to, invades and induces oncogenic and inflammatory responses to stimulate growth of CRC cells through its unique FadA adhesin. FadA binds to E-cadherin, a cell-cell junction molecule and a cancer suppressor, leading to activation of β-catenin-regulated transcription, and differential regulation of the inflammatory, oncogenes and Wnt gene expressions. Stimulation of the inflammatory responses was dependent on FadA invasion while activation of the Wnt and oncogenes was not. The FadA-binding site on E-cadherin is mapped to an 11 amino acid region. A synthetic peptide derived from this region abolishes FadA-induced CRC cell growth and activation of the inflammatory, oncogenes and Wnt gene expressions. FadA levels in the colon tissue from patients with benign polyps (adenomas) and malignant carcinomas are >10-100 times higher compared to normal individuals. The increased FadA expression in CRC correlates with increased expression of Wnt and inflammatory genes. Our study suggests an infectious nature of CRC and unveils a mechanism by which *Fn* can drive CRC. In addition, we identify FadA as a potential diagnostic and therapeutic target for prevention and treatment of CRC.

ORAL BACTERIA AND SYSTEMIC DISEASE: *P. GINGIVALIS* CAN CAUSE WHAT?!

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Porphyromonas gingivalis is a dysbiotic oral pathogen involved in the destruction of periodontal tissues. While at first glance oral infections may seem exquisitely tissue specific, increasing evidence implicates a role for oral bacteria such as *P. gingivalis* in serious systemic conditions including diabetes, coronary artery disease, preterm delivery of low birth weight infants, obesity, Alzheimer's disease, rheumatoid arthritis and cancer. A conceptual framework for these associations is provided by the frequent occurrence of *P. gingivalis* bacteremia, and consequent migration of the organism to remote sites where tissue homeostasis and/or inflammatory responses are disrupted. In placental tissue *P. gingivalis* can be detected more frequently in women with chorioamnionitis as compared to normal pregnancies. *P. gingivalis* can invade trophoblasts in culture and the outcomes of the *P. gingivalis*-trophoblast interaction have implications for the maintenance of pregnancy. *P. gingivalis* inhibits proliferation of trophoblasts by inducing a G1 arrest which is associated with decreased expression of cyclin D and of CDKs 2, 4 and 6. In addition, levels of CDK inhibitors p15, p16, p18 and p21 are increased following *P. gingivalis* infection. Trophoblasts halted in the G1 phase become apoptotic, and apoptosis is accompanied by an increase in the ratio of Bax/Bcl-2 and increased caspase activity. In the short term, *P. gingivalis* disrupts signaling through the MAP kinase pathway and increases the production of the cytokines IL-1β and IL-8. Epidemiologically, *P. gingivalis* is also associated with pancreatic cancer and oral squamous cell carcinoma (OSCC). Moreover, OSCC surfaces harbor significantly higher levels of the organism compared to the contiguous healthy mucosa. In oral epithelial cells *P. gingivalis* is strongly anti-apoptotic primarily through activation of Jak1/Akt/Stat3 signaling which controls intrinsic mitochondrial apoptosis pathways, and by secreting a nucleoside diphosphate kinase which can function as an ATPase and prevent ATP-dependent apoptosis mediated through the purinergic receptor $P2X_7$. In concert with suppression of apoptosis, *P. gingivalis* can accelerate progression through the S-phase of the cell cycle by manipulation of cyclin/CDK activity and reducing the level of the p53 tumor suppressor. In OSCC cells themselves *P. gingivalis* infection activates the ERK1/2-Ets1, p38/HSP27, and PAR2/NF-κB pathways to induce pro-matrix metalloproteinase (MMP)-9 expression. MMP-9 degrades basement membrane and extracellular matrix, which promotes carcinoma cell migration and invasion, thus allowing carcinoma cells to enter the lymphatic system and blood vessels for dissemination and metastatic growth at remote sites.

ORAL ANAEROBES IN HEALTH AND DISEASE: 2014 HIGHLIGHTS

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Anaerobes constitute a significant part of the human oral microbiome. Their ability to colonize and survive in an environment, where remarkable changes occur during early childhood and adolescence, is fundamental for oral homeostasis. But if the oral homeostasis is disturbed by a variety of factors, such as unbalanced diet, immunosuppression, or antibiotic therapy, oral infections may occur with serious systemic consequences.

It has been recognized that oral infections, especially marginal and apical periodontitis, may affect the course and pathogenesis of a number of systemic diseases, such as infective endocarditis, cardiovascular disease, bacterial pneumonia, diabetes mellitus, and low birth weight. The purpose of this review is to evaluate the 2014 status of oral anaerobes as a causal factor for local and systemic diseases. Three mechanisms for a systemic impact have been proposed: (i) spread of mixed anaerobic infection from the oral cavity as a result of transient bacteremia, (ii) circulating oral microbial endo- and exotoxins, and (iii) immunocomplexes (oral antigens & antibodies) which give rise and add on to a variety of acute and chronic inflammatory reactions with increased release of highly active mediators. Proposed evidences and mechanisms of the above odontogenic systemic diseases are given. Some related highlights from the 2014 European Oral Microbiology Workshop in Aarhus, Denmark, will be summarized.

HIGHLY DIVERSE ROOT CANAL MICROBIOTA IN CHRONIC APICAL PERIODONTITIS ACCORDING TO ILLUMINA SEQUENCING

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Chronic apical periodontitis (CAP) is an inflammatory process in the surrounding area of dental root. CAP is a frequent (prevalence 30%...70%) but poorly understood condition that has considerable effect on patient's life quality. Etiology and pathogenesis of CAP have not been finally elucidated and the current treatment options are not always successful. Therefore additional studies are urgently needed.

Objective. To reveal root canal microbial communities in case of chronic apical periodontitis applying Illumina sequencing.

Methods. Study group included 14 antibiotic naive patients—5 patients with primary (pCAP) and 5 patients with secondary apical periodontitis (sCAP), and 4 patients with periapical abscess (PA). Microbiome was profiled using Illumina HiSeq 2000 sequencing of the V6 region of 16S rRNA gene.

Results. All subjects harboured highly polymicrobial communities in their root canals. One sample contained 5-8 (mean 6.5) phyla of bacteria. The most numerous were Firmicutes ja Bacteroidetes but also Actinobacteria, Fusobacteria, Proteobacteria, Spirochaetes, Tenericutes and Synergistetes were present. One sample contained 30-70 different bacteria. Anaerobic bacteria predominated both in pCAP and sCAP patients but the communities were individually different. In sCAP patients more Gram-positive bacteria were found. *Enterococcus faecalis* (an important reason for treatment failure) were found from sCAP patients only. One PA sample displayed significantly higher proportion of Gram negative *Proteobacteria* than all other samples (at the expense of *Janthinobacterium lividum* that is able to produce anti-bacterial, anti-viral, and anti-fungal compound violacein).

Conclusions. Illumina sequencing reveals highly polymicrobial individually distinct communities in dental root canals in cases of both pCAP and sCAP. Trend towards Gram positive bacteria exists in cases of sCAP.

730 *SATELLITE SYMPOSIUM I:* A NEW LOOK AT ANAEROBES IN THE CLINICAL LABORATORY

SATI-1

A NEW LOOK AT ANAEROBES IN THE CLINICAL LABORATORY

The identification (ID) and susceptibility testing (AST) of anaerobes in the clinical laboratory can be difficult and time consuming. Routine laboratories may not have the expertise to provide anaerobe ID & AST, yet the clinical significance of these organisms is increasingly recognized due to the diseases they cause and because their susceptibility patterns are not always predictable.

ID of anaerobes has generally relied on phenotypic testing, made difficult by the slow growth and often biochemically inactive behavior of the organisms. This leads to long turn-around times for results, often hindering the selection of appropriate antibiotic treatment. New technologies being implemented can improve ID accuracy and reduce the time to results. The introduction of MALDI-TOF mass spectrometry provides an accurate, cost effective method to significantly reduce the difficulty and time required to obtain an ID of these organisms.

As with the ID, AST of anaerobes is generally not performed routinely due to its complexity. However, susceptibility patterns may be variable with these organisms and AST is becoming more important to aid in treatment decisions. Without laboratory results on individual isolates, clinicians must rely on published data to predict whether an antibiotic will be effective.

While routine AST of anaerobes may not be required for all isolates, particular species, infection sites, patient populations, etc. may be considered for testing. For example, isolates from patients with surgical site infections, endocarditis, osteomyelitis, joint infections, and bacteremia should be considered for testing. Highly virulent pathogens such as Bacteroides, Fusobacterium, and Clostridium should be considered for testing. Antimicrobials effective against anaerobes include beta-lactams, combinations of beta-lactams and beta-lactamase inhibitors, metronidazole, chloramphenicol, clindamycin, macrolides, tetracyclines, and fluoroquinolones. The susceptibility to these agents must be monitored periodically to check for developing resistance.

A brief discussion of current recommendations and methods for anaerobe testing will be presented. The spectrum of efficacy, antimicrobial resistance mechanisms, and resistance patterns against these agents are described.

845 *SESSION VI:* BIOFILMS IN ANAEROBIC INFECTIONS

MICROBIAL BIOFILMS AND COLON CANCER

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The colonic microbiome is proposed to contribute to the initiation and progression of human colon cancer. To address this hypothesis, we are prospectively studying colon cancer samples and parallel normal colon mucosa from colon cancer hosts and colon biopsies (right and left) of healthy controls undergoing screening colonoscopy. Microbial analyses include structural microbiology (Carnoy's fixed tissues to visualize mucosal bacteria using bacterial 16S rRNA labeled probes) as well as 454 pyrosequencing and microbiology/qPCR for specific putative oncogenic bacteria. In right colon cancers (defined as proximal to the hepatic flexure) examined to date, a marked biofilm is associated with both the colon cancers and 100% of normal tissues from the cancer host. In contrast, only 13% of left colon cancers (distal to the hepatic flexure) and their parallel cancer-free tissues exhibit a marked biofilm. Among colonoscopy tissues, ~10% of normal biopsies display limited biofilm formation. FISH analyses revealed bacterial invasion of all biofilm+ tumors and a subset of biofilm+ normal tissues from colon cancer hosts. Histologically normal biofilm+ tissues from colon cancer hosts and colonoscopy controls exhibit a significant increase in proliferation relative to biofilm(-) specimens. Principle component analyses of mucosal 16S sequencing data supports the hypothesis that the colon mucosal microbiota communities develop progressive, dysbiotic changes moving from normal colon in healthy adults (colonoscopy) to colon cancer. Our data identify the mucosal microbiota organization, as opposed to its specific composition, as a critical factor accelerating oncogenic progression in right- and some left-sided colon cancer. Colon mucosal biofilm detection may predict increased risk for development of sporadic colon cancer.

GARDNERELLA VAGINALIS BIOFILMS IN BACTERIAL VAGINOSIS

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Bacterial vaginosis (BV) is a dysbiosis characterized by a change in the normally *Lactobacillus*-dominant vaginal microbiota to one with greater microbial diversity, particularly with regard to anaerobic organisms. BV is associated with several important adverse health outcomes, including premature birth and acquisition and transmission of sexually transmitted diseases. Diagnostic tests for BV have suboptimal sensitivity and specificity, and treatment strategies including local and systemic antibiotics are limited by frequent recurrence of the condition. The association of the facultative anaerobe *Gardnerella vaginalis* (GV) with BV has been noted since the 1950s, but a specific role for this organism in BV has not been demonstrated conclusively. GV forms tenacious biofilms both *in vitro* and at the vaginal mucosal surface during BV. This biofilm has been hypothesized to be an important factor in BV initiation, recurrence, and transmission. In recent work, we have demonstrated that extracellular DNA (eDNA) represents an important structural component of the GV biofilm matrix. Enzymatic disruption of eDNA destabilizes GV biofilms and is synergistic with the anti-GV antibiotics metronidazole and clindamycin. Using newly developed murine models, we have investigated the importance of biofilm growth to the establishment of colonization and tracked GV ascension to the uterus and placenta. The development of DNase gels with improved physiochemical characteristics for early preclinical trials is underway, and such biofilm-targeting agents represent a promising new avenue in BV research.

CLOSTRIDIUM DIFFICILE BIOFILMS *(IN VITRO)*

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Clostridium difficile infection (CDI) is a leading cause of healthcare-associated morbidity and mortality around the world. CDI typically appears after a patient on antibiotic therapy ingests spores of a toxin-producing *C. difficile* strain. It is not known whether, during infection, *C. difficile* forms a community in association with the GI tract mucosa. Locating and characterizing any such communities within the GI tract could reveal important novel features of CDI pathogenesis, including mechanisms of colonization, immune evasion, and persistence and relapse. This knowledge could also have a significant impact on future treatments. To address these questions, we analyzed *C. difficile* communities formed *in vitro* using a monoculture, and on the GI tract mucosa, during infection in a mouse model. We characterized community formation *in vitro* by growing *C. difficile* on a polycarbonate filter. We found that *C. difficile* forms biofilm-like communities, containing spores and an extracellular matrix possessing DNA, polysaccharide and protein, including toxin. Biofilm cells are functionally distinct from conventionally grown cells: cells possess greatly elevated resistance to the antibiotic metronidazole, and spores isolated from the biofilm germinate with significantly reduced efficiency. Taken together, these data suggest that *C. difficile* cells and spores in biofilms have specialized properties that may facilitate infection. We also analyzed *C. difficile* communities *in vivo*, using a mouse model of CDI. Using 16s rDNA sequencing to preliminarily analyze the GI tract microbiome community population structure and to design probes for *in-situ* hybridization (FISH) analysis of tissue sections, we identified *C. difficile*-containing communities within 2 days after infection and until at least 8 days, on or close to the cecal and colonic mucosal surfaces. *C. difficile* is only a minority member of these communities; members of the Enterobacteriaceae and Bacteroidaceae families, the Lactobacillus and Enterococcus genera, and members of Clostridium clusters XIVa and XIVb are also readily detected. We intend to use these results to characterize *C. difficile*-containing communities in the host in greater detail, and to elucidate relationships between community structure, and the progression of disease and resistance to treatment.

THE MICROBIOME IN ORAL HEALTH AND DISEASE

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Smoking has been established as a substantial risk factor for the development of caries, oral cancer and periodontal disease. The odds of developing oral cancer and periodontitis are 16-fold, and that of caries is 10-fold. With 1.2 billion smokers worldwide, oral disease in smokers is an enormous health burden. Many mechanisms, including alterations in numerous host-response factors, have been proposed to account for these effects, however, a consistent explanation is not available. Bacteria in dental plaque are *primary* etiological agents of these diseases. It is possible that smoking modifies this community, leading to many of the observed changes. Exploring these changes is an important step in identifying the mechanism of action of smoking on oral tissues. Biofilms are important for maintaining oral health, however disruptions in these communities play an integral role in the susceptibility and progression of disease. With the emerging evidence that smoking alters the subgingival ecosystem, we investigated the effect of smoking on the subgingival microbiome. By combining cross-sectional as well as longitudinal study designs with metagenomic and metatranscriptomic approaches, we have begun elucidating the mechanism of action of smoking on the oral bacteria. Our data demonstrate that smoking may contribute to the creation of high-risk microbial communities even in states of health, by altering the gene content of these biofilms. These genomic differences may result in differences in gene expression and inflammatory potential, thereby contributing to future disease in this high-risk population.

1035 ***SESSION VII:*** **DIAGNOSTIC & LABORATORY TECHNIQUES**

OSCILLIBACTER WHO? WHAT SHALL WE DO WITH THESE NEW NAMES?

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One of the most important reasons for fully identifying anaerobes recovered from blood has always been to locate the primary infection site, since most of these infections are caused by normal flora isolates escaping from a primary colonized site. Since the advent of 16S rRNA gene sequencing, as well as other molecular identification methods and MALDI-TOF MS, many "new" organisms have appeared—some as just sequences from DNA segments in stool and environmental samples, but many from important infections including blood, wounds and other conditions. These organisms have always been here, but as nameless germs they could not attract any attention, nor could we study them. Now that increasing numbers of labs are sequencing and describing new organisms, the challenge is finding the best approach for the laboratories to report and for clinicians to use this information. Knowledge of the normal flora has been important for characterizing infections, yet now we are seeing new names that are unknown as colonizers in any body site. So what in the world is *Oscillibacter ruminatum* and how did it get into bloodstream infections? A quick read of the original description gives the first isolate as coming from the rumen of native Korean cattle. Fortunately, the antimicrobial susceptibilities are provided in the bacteremia paper so the clinician could choose the best antimicrobial agent for treating the infection and could infer from the clinical description of the patients that *Oscillibacter* is a previously undescribed human gut organism as well. Subsequent research has shown that *Oscillibacter* is found in elevated numbers with decreased lactobacilli in gut flora of mice fed high fat diets, with resulting metabolic dysfunction, increased mesenteric fat inflammation and increased gut permeability. Could *Oscillibacter* play a role in the cause of the leaky bowel or be a result? A very intriguing question for which there is no answer at this time. As other organisms are described and named, additional research will be undertaken. Until more laboratories start to report their new findings, thus giving clinicians the opportunity to become familiar with the new genera and species, the new names will be a challenge. Ultimately, the body of knowledge of the microbiome, infectious agents and their mechanisms of action will increase our understanding of infectious diseases and improve our approach to treating patients.

EUROPEAN EXPERIENCE WITH MALDI-TOF MS IN THE FIELD OF ANAEROBES

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After the enthusiasms of routine microbiology laboratories in the 1960s to find and prove the role of different anaerobic bacteria in severe infections of humans, by the 1990s most of them started to simplify reported culture results as "mixed anaerobes" both in Europe and in the US. Important isolates were referred to the few reference laboratories, where the identification of anaerobic bacteria was carried out by different time-consuming and laborious phenotypic and DNA-based molecular methods. Following the successful adaptation of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the routine laboratory identification of bacteria, the creation of an extensive database has been initiated to facilitate the use of this method for the identification of anaerobic bacteria as well. Not only frequently isolated anaerobic species, but also newly recognized and taxonomically rearranged genera and species can be identified by using direct smear samples or whole cell protein extraction, and even phylogenetically closely related species can be identified correctly by means of MALDI-TOF MS. Two systems, the MALDI Biotyper (Bruker) and the Vitek MS (BioMerieux), are available at the present time as easy-to-use MALDI-TOF MS instruments. The two systems are different how the species- or genus-level identification is performed from the measured mass spectra and for the databases of the two systems for anaerobes. In the past three years an extensive database development is going on in European laboratories dealing with anaerobes on high level. Pre-analytical factors such as use of different media, exposure to oxygen, as well as time of incubation in the anaerobic environment were evaluated. Two study groups of ESCMID, ESGAI, and ESGEM initiated the European Network for the Rapid Identification of Anaerobes (ENRIA) with the aim to fill the gaps in the database of clinically important anaerobes. Further data are available about the use of this method for typing of different anaerobes as well as to determine carbapenem hydrolysis. There is a possibility for the direct identification of anaerobes in positive blood cultures. This rapid and cost-effective method has already revolutionized anaerobe bacteriology in Europe.

SVII-3

ANIMAL MODELS FOR *CLOSTRIDIUM DIFFICILE* INFECTION

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Clostridium difficile is the leading cause of nosocomial diarrhea and colitis in healthcare facilities. Over thirty-five years after the recognition of *C. difficile* as the cause of antibiotic-associated colitis, we still have significant gaps in our knowledge of the pathogenesis of *C. difficile* infection (CDI). Moreover, the clinical spectrum of CDI has evolved and currently infections are increasingly difficult to treat because of the high rate of disease recurrence after antibiotic therapy, leaving few treatment options for patients. The recent development of multiple animal infection models to study the interactions between *C. difficile*, the host and the microbiota are providing novel insight into the mechanisms of pathogenesis and transmission that should guide the development of therapies and intervention measures. In this session, I will review the historical development of animal models of CDI and summarize what insights have been gained with regards to the pathogenesis of infection. Remaining gaps and future challenges will be highlighted, pointing the way to future research on this important nosocomial infection.

1350 SESSION VIII: THE CARE AND FEEDING OF OUR INTESTINAL MICROBIOME

FUNCTIONAL DAIRY FOODS AND HUMAN INTESTINAL MICROFLORA

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Functional foods are foods consumed for specific health purposes, because of their constitution in nutrients or other substances which may confer health benefits action for their host. Although they are known from thousands of years ago, Metchnikoff during the years 1900, in his Thesis correlated the intake of large quantities of Bulgarian fermented milk with the longetivity of the Caucasian people. Many functional foods now exist in various countries. Internationally, they are grouped in 3 main classes: probiotics, prebiotics, and synbiotics. A plethora of healthful effects have been attributed to the probiotic lactic acid bacteria, The contribution of biotechnology has been very important in the selection of new strains, improvement of specific functional properties, nutritional improvement of foods, and finally, improvement of sensory and textural qualities of the final product. However, the capital role of functional foods is focused on the stimulation of the host immune system and preservation of the microbial intestinal balance *via* the "barrier effect". It is, then, obvious, that the probiotic approach will help to determine the role of the bacterial species, as well as ingredients promoting their growth in the gastrointestinal tract. Clearly, the beneficial potential of the human microflora is qualified together with the deficiencies in the gut flora, as a tool having a protecting effect upon the gut microbiota. *Lactobacillus*, which are facultative anaerobic or aerobic rods, are an important part of the human microflora by inhabiting various organs without usually exerting any pathogenic effect. Stress influence seems to provide the gastrointestinal microflora with putrefactive bacteria and, especially, increase *C. perfringens* numbers. Gastrointestinal infections, bacterial or viral diarrhoeas disease, preudomembranous colitis and antibiotic-associated diarrhoea have been treated successfully by using some pharmaceutical probiotics as *S. boulardii, L. casei GG, L. acidophilus*, and *E. faecium*. Conversely, prebiotics alter the bacterial composition of the gut, not by adding bacteria, but by changing the type of substrate provided to the existing mixture. In this vein, it is understandable that research in the field of development of novel functional foods which lies in modification of the activity of the gastrointestinal tract by use of probiotics, prebiotics, and synbiotics seems to be one of the most promising areas.

NEW ANTIMICROBIAL STRATEGY BASED ON INHIBITION OF PATHOGEN'S CELL-TO-CELL SIGNALING AND CONSECUTIVE VIRULENCE FEATURES EXPRESSION BY PROBIOTIC SIGNAL MOLECULES

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The overuse/misuse of antibiotics induced the present high level of antibioresistance (AR) which is confronting mainly the medical world, but even other social sectors, with an indirect impact on public health. The problem of AR is amplified by the biofilm associated infections and the tolerance of biofilm embedded cells to antimicrobials. Thus, the interest of researchers has been attracted toward complementary / alternative therapeutical strategies, which can be used in order to replace or potentate the effect of regular drugs. Among these novel antimicrobials it has been identified an ecological strategy, based on the use of signal biomolecules able to break off the pathogen's signaling pathways by quorum sensing (QS) mechanism. The probiotics already have demonstrated benefits on human health, as mono-strain or multistrain products which are improving health by either supporting host physiology or by their antiinfectious barrier. The interaction of probiotics with the host is complex, involving probiotic surface structures and soluble metabolites active on normal microbiota and host cells, with antiinfectious and immunomodulatory effect too. More recently it has been proved that the probiotic products can have dual action and could turn from 'generally recognized as safe' harmless bacteria, into detrimental ones in some cases (infectious, inflammatory, metabolic diseases). So, it is important to know which are the intimate mechanisms of action and respectively, the benefits and side effects of each component. It is proved that the probiotic cells are producing antimicrobial substances, but also small molecules of QS inhibitors which are interfering with the QS mechanism of pathogens and their virulence genes expression. The intercellular signaling is a relatively recent field of research, very promising for a new perspective on antiinfectious therapy management. In this presentation, it will be discussed the pathway for fight against pathogens by soluble probiotic molecules targeting the QS circuits, so, a new target and an intelligent strategy, without any risk of inducing resistance or other side effects.

1440 ***SESSION IX:*** **CLOSTRIDIUM SPP: HEALTH AND DISEASE**

THE OPPORTUNISTIC PATHOGEN *CLOSTRIDIUM SEPTICUM*

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Purpose: To review key epidemiological and pathophysiological features of human disease caused by *Clostridium septicum*.

Methods: This oral presentation brings together published data about infections caused by *C. septicum* and the state of knowledge regarding disease pathogenesis. New data will be presented from a surveillance study conducted using culture and molecular detection (real time PCR) to quantify the point prevalence of *C. septicum* in human fecal specimens obtained from a single academic medical institution in Michigan. Fecal specimens were obtained from 161 asymptomatic community-dwelling adults and 192 hospitalized patients with diarrhea (but negative for *C. difficile*). Real time PCR was conducted to amplify the *csa* gene encoding the *C. septicum* pore forming alpha toxin gene. A BLAST search for evidence of *C. septicum* 16S rRNA gene sequence was conducted against a library of partial 16S rRNA sequences obtained from 338 human stool samples (a convenience sample that included 169 of the samples used for the culture- and *csa*-based investigations above).

Results: No evidence for *C. septicum* carriage was identified among these stool samples based on culture, PCR for *csa* gene, or based on the 16S rRNA BLAST search.

Conclusions: *C. septicum* is an uncommon but important cause of highly lethal infections, particularly in those with pre-existing bowel disease such as cancer or colitis. Immunocompromised patients, especially those with mucositis or recent chemotherapy are also at increased risk, and infections of the female reproductive tract during or soon after pregnancy are reported. Point prevalence appears to be low, suggesting that *C. septicum* is not a normal commensal in humans but an opportunistic pathogen whose capacity to cause disease reflects the coincidental occurrence of transient carriage and an enhanced host susceptibility.

Other: Support for this study was provided by the National Institutes of Health, grant 5U19AI090871. Dr. Aronoff has served as an advisor for the Gut Check Foundation, a non-profit organization that promotes education and research into the prevention and treatment of *C. septicum* infections.

LIFE-THREATENING TOXIN MEDIATED CLOSTRIDIAL INFECTIONS

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Devastating Clostridial infections—including tetanus, botulism, and gas gangrene—have been recognized for centuries and are well described even in the ancient literature. Over the course of the last 150 years most of the reported cases of necrotizing or histotoxic clostridial infections have been associated with war time injuries and civilian injuries due to gunshots and compound fractures. In more recent times, as civilization and medical care have evolved, a variety of clinical presentations have emerged.

Recently gas gangrene caused by *Clostridium perfringens* has been associated with multiple medical procedures and underlying illnesses such as liver transplantation, Caesarian delivery, ovarian carcinoma, colonoscopy for benign polyps, hydadiform mole of the uterus, intravenous catheter insertion site, following *Clostridium difficile* colitis, diabetic foot ulcer, intramuscular injection of Vitamin B-12, IM injection of sodium diclofenac, mesenteric lymph node biopsy, following hernia repair, iliac crest bone donor site for treatment of a non-union of a clavicular fracture and in association with a *Toxoplasma gondii* infection of the uterus. Similarly, *C. septicum* infections have not only been associated with traumatic injuries in normal hosts, but also in patients with neutropenia and gastrointestinal lesions to name a few. *C. perfringens, C. septicum, C. novyii, C. histolyticum,* and *C. sordellii* have become less common causes of infection in wartime due to rapid evacuation and vascular re-construction. Still, these microbes continue to be associated with trauma associated with natural disasters such as earthquakes, hurricanes, and tornados. Finally, an unusual strain of clostridia, *C. fallax* has been reported as a cause of small bowel necrosis and death in a 16 year old healthy boy.

Specific mechanisms of pathogenesis will be discussed in terms of mechanisms of shock and organ failure and vascular injury. Management issues are complex, however appropriate antibiotics and surgical intervention remain the key elements. Despite contemporary management, morbidity, and mortality are high and clearly novel new therapies directed against specific toxins and the dramatic host response to infection are sorely needed.

SPORES OF *CLOSTRIDIUM* ENGINEERED FOR CLINICAL EFFICACY AND SAFETY CAUSE REGRESSION AND CURE OF TUMORS *IN VIVO*

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The ability of intravenously injected clostridial spores to infiltrate and thence selectively germinate in the hypoxic regions of solid tumors has been used to deliver prodrug converting enzymes to tumors of selected animal models, and shown to result in beneficial effects. This approach has been termed Clostridial-directed Enzyme Prodrug Therapy, or CDEPT. We have now addressed and solved previous shortcomings, namely the poor kinetics of CB1954 prodrug activation by the nitroreductase (NTR) enzymes and the instability of the added *trans* gene. Accordingly, we have identified, characterised and deployed a much improved NTR enzyme that has a Km CB1954 value that can be exceeded by the clinically achievable serum concentration of the prodrug. Secondly, we have localized expression cassettes to the chromosome using double crossover homologous recombination, and have constructed stable strains that lack both antibiotic resistance markers and any mechanism for transfer of the heterologous sequence. Concomitant with the insertion of the *trans* gene, we have introduced a specific form of disablement (a requirement for exogenous uracil through mutation of the *pyrE* gene) which serves to severely limit growth of the *C. sporogenes* strains in the environment in the event of accidental release from a clinical setting. The use of the created strain in a mouse xenograft model of human colon carcinoma in combination with CB1954 demonstrated substantial tumor suppression with several animals being cured. Our approach to constructing stable, disabled, antibiotic marker-free strains of *Clostridium* expressing heterologous genes represents a platform for future efforts to build upon, as it provides a tumor-specific delivery system for the development of safe and effective gene therapies.

CLOSTRIDIUM SORDELLII INFECTIONS: INSIGHTS INTO THE PATHOGENESIS OF DISEASE

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Clostridium sordellii is a spore-forming bacillus known to cause myonecrotic and gangrenous-like infections in animals and humans. *C. sordellii* infections are associated with childbirth and routine gynecological procedures. Infections following medically-induced abortions, intravenous drug use and traumatic wound injury have also been reported. *C. sordellii* infections pose difficult clinical challenges and are usually fatal (~ 70%). Fatal infections are characterized by an absence of fever, mild tissue inflammatory response, a massive capillary leak syndrome, and a severe leukemoid reaction (LR). Patients ultimately succumb to a toxic shock syndrome hours to days following the initial symptom display. We recently established a murine model of *C. sordellii* myonecrosis to investigate clinical features associated with human cases of infection. Here, female C57BL/6 mice were inoculated with 0.5, 1.0 and 2.0 x 10^6 CFU of *C. sordellii* (ATCC 9714 type strain) in the right upper thigh muscle. The survival rates and timing of mortality was inoculum dose-dependent. As in human infections, severe edema, little inflammation and moderate leukocyte infiltration was observed at the site of infection. In addition, mice also developed increased white blood cell counts, and the timing at which circulating WBC counts peaked paralleled the severity of infection. Histopathology analysis of infected tissues showed muscle tissue damage, edema, and neutrophil infiltration. Increases in circulating G-CSF and IL-6, but not TNFα- or IL-1β, were also observed in the serum of animals infected with lethal doses of *C. sordellii*. Our studies now provide a well-suited model for evaluating intramuscular *C. sordellii* myonecrosis and may be considered as a novel and convenient model for investigating host-pathogen interactions and systemic manifestations of *C. sordellii* soft-tissue infection.

SIX-5

IDENTIFICATION OF THE HOST RECEPTOR FOR *CLOSTRIDIUM PERFRINGENS* TPEL TOXIN INDICATES A TWO-RECEPTOR MODEL OF CLOSTRIDIAL GLYCOSYLATING TOXINS

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Clostridial glycosylating toxins are major virulence factors of various species of pathogenic *Clostridia*. Prototypes are *Clostridium difficile* toxins A and B, which cause antibiotics-associated diarrhea and pseudomembranous colitis. The current model of the toxins' action suggests that receptor-binding is mediated by a C-terminal domain of combined repetitive oligopeptides (CROP). This model is challenged by the glycosylating *Clostridium perfringens* TpeL toxin that is devoid of the CROP domain but still intoxicates cells. Using a haploid genetic screen, we identified the host cell receptor for the TpeL toxin. Receptor-deficient cells were not intoxicated by TpeL but regained sensitivity towards the toxin after ectopic expression of the receptor. By plasmon resonance spectroscopy a K_D value of 23 nM was determined for binding of TpeL to its receptor. We further identified that the C-terminus of TpeL (residues 1335-1779) represents the receptor-binding domain (RBD) of the toxin. RBD-like regions are conserved in all other clostridial glycosylating toxins preceding their CROP domain. We found that CROP-deficient *C. difficile* toxin B is toxic to cells, depending on the RBD-like region (residues 1349-1811) but does not share the same receptor as TpeL for cell entry. Our data indicate the presence of a second, CROP-independent receptor-binding domain in clostridial glycosylating toxins and suggest a two-receptor model for the cellular uptake of clostridial glycosylating toxins. Thus, our study offers a new perspective in the understanding of the pathogenicity of this group of clinically important toxins. Identification of the cell membrane receptor and/or the corresponding receptor-binding domains within toxins are groundbreaking steps for the development of anti-toxin strategies.

1610 ***SESSION X:*** **WHOLE GENOME SEQUENCING**

GENOMIC INVESTIGATIONS OF THE MATERNAL MICROBIOME DURING PREGNANCY AND ITS ASSOCIATION WITH PRETERM BIRTH

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Preterm birth remains a pressing, intractable problem worldwide. Subclinical maternal infection and inflammation during pregnancy is thought to contribute to preterm birth in many cases, especially those occurring at early gestational ages that confer the greatest risk for neonatal morbidity and mortality. Increasing evidence suggests that cryptic infectious or inflammatory states in pregnant women may largely be driven by members of the host microbiota—including commensal microbes comprising perturbed, "unhealthy" communities—rather than by an exogenously acquired pathogen. Conventional laboratory approaches often fail to identify affected women, or to predict the outcome of preterm delivery.

Increasingly, a variety of molecular approaches are being brought to bear on this problem, including 16S rDNA amplicon sequencing, shotgun sequencing, and whole genome sequencing, among others. These investigations have implicated specific taxa, including anaerobic species that typically inhabit one or more body niches (e.g., the vagina, oral cavity and distal gut). Large-scale, longitudinal, prospective genomic studies of the pregnancy-associated microbiome have been undertaken recently. These investigations hold promise for characterizing, at high temporal resolution, the compositional and functional dynamics of the maternal microbiome, including potential stereotypic patterns that may be associated with the outcome of preterm birth.

URINE IS NOT STERILE: NEW TOOLS LEAD TO NEW HYPOTHESES

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The assumption that, in health, the female urinary system does not have a resident microbial community has limited consideration of the spectrum of potential etiologic explanations and/or clinical treatments for benign urologic disorders. This "sterile" paradigm is inconsistent with emerging information from the Human Microbiome Project, which has successfully identified profound effects of microbiota from various body sites on a wide range of human diseases.

Complementary high throughput DNA sequencing-based approaches and expanded quantitative urine culture (EQUC) techniques have shown that the "sterile paradigm" is wrong, revealing compelling evidence that the female urinary tract possesses its own unique microbiota. With the discovery of the female urinary microbiota (FUM), we have begun to re-examine fundamental assumptions concerning urologic health and disease.

In this talk, I will describe several current investigations that more fully define the composition of the FUM, especially in women with overactive bladder (OAB). OAB is a common lower urinary tract disorder characterized by the sudden urge to urinate, often with involuntary loss of urine.

I will show that the FUM is often composed of anaerobes that do not grow under the standard clinical laboratory protocol, and thus have been overlooked as potential etiologic agents of chronic lower urinary tract disorders. I will show that urinary bacteria associated with OAB are distinct both from urinary bacteria of women with no lower urinary tract symptoms, and from the bacteria that cause overt clinical urinary tract infection (UTI). I will present evidence that the FUM is linked to certain clinical symptoms (such as the number of UUI episodes per day) and clinical outcomes (such as risk of post-instrumentation UTI), suggesting that the FUM has clinical implications. Finally, I will highlight exciting novel investigations that we believe will enhance our understanding of etiology, prevention and treatment of benign urologic disorders.

1710 *SESSION XI:* ANTIMICROBIALS AND RESISTANCE

THE DRUG PIPELINE FOR ANAEROBIC INFECTIONS

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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, biapenem) or new (e.g. ceftolozane) beta-lactam agents in combination with new beta-lactamase inhibitors (avibactam, MK-7756, RPX7009). Most are targeted towards their activity against new aerobic gram-negative MDROs (multiply drug resistant organisms). These compounds have or are undergoing clinical evaluations in the therapy of intra-abdominal infections. Several older fluoroquinolones (sitafloxacin and moxifloxacin + metronidazole) are under active clinical investigation in intra-abdominal infections.

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

GSK2251052, a novel boron-containing leucyl-tRNA synthetase inhibitor has been evaluated *in vitro* against a wide variety of anaerobes but its clinical trial was withdrawn suggesting that it is unlikely to be further developed.

ANTIMICROBIAL SUSCEPTIBILITY TESTING IN EUROPE

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During several years, an increasing resistance to different antimicrobial agents in anaerobic bacteria has been observed. Anaerobic bacteria are naturally resistant to certain antibiotics, including the aminoglycosides and the earlier quinolones, and many anaerobes now exhibit resistance to several betalactam agents as well. Resistance to betalactam agents is most often caused by production of betalactamases, enzymes that inactivate betalactam compounds by hydrolysis. Changes in the penicillin-binding proteins or blocked penetration of drug into the active site via alteration of the bacterial outer membrane pores are also decreasing the susceptibility to betalactam agents. Resistance is more common in Gram-negative than in Gram-positive anaerobic isolates. Patterns of susceptibility vary in different geographic areas and even in different hospitals in the same city, depending on antibiotic-prescribing practices. Four groups of drugs are active against almost all anaerobic bacteria of clinical significance: nitroimidazoles, carbapenems, chloramphenicol, and combinations of betalactam drugs with a betalactamase inhibitor (ampicillin/sulbactam, amoxicillin/clavulanic acid, piperacillin/tazobactam). Antimicrobial susceptibility testing of anaerobic bacteria has been made in microbiological laboratories in Europe since 1970. Different methods have been used and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) has been involved in the development of standardized methods during the last 20 years. Two groups, ESCMID Study Group for Anaerobic Infections (ESGAI) and ESCMID Study Group for *Clostridium difficile* (ESGCD), together with European Committee on Antimicrobial Susceptibility Testing (EUCAST) are now actively working with the project. New data including newer antimicrobial agents, breakpoints, resistance mechanisms, epidemiology and clinical implications will be presented at the 24th European Congress of Clinical Microbiology and Infectious Diseases, May 9-13, 2014 in Barcelona, Spain. The most important findings will then be discussed at the Anaerobe 2014 meeting, June 30, 2014 in Chicago, Illinois, USA.

EMERGENCE AND EVOLUTION OF AN INTERNATIONAL CLONE OF MULTIRESISTANT *BACTEROIDES FRAGILIS* ISOLATES

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During our investigations of the antibiotic resistance mechanisms of *Bacteroides spp*. in the frame of ESGAI, a particular set of genetic constitutions (*cfiA*, *nimB*, IS*1186* and IS*4351*) proved to be frequent among carbapenem and metronidazole-resistant *B. fragilis* strains from the UK, Sweden, France, Hungary ,and the USA. 7 such *B. fragilis* strains were characterized in most detail. Antibiotic susceptibilities were recorded by a gradient technique (Etest, bioMerieux) (piperacillin, piperacillin/tazobactam, cefoxitin, meropenem, clindamycin, moxifloxacin, metronidazole, teracycline and tigecycline). Resistance gene carriage (*cepA*, *cfxA*, *cfiA*, *nimB*, *ermF*, *tetQ* and *tetX*) and CTn and insertion sequence (CTnDOT, IS*1186* and IS*4351*) element contents were recorded by PCR, and we detected the nucleotide sequence environments of the *cfiA* and *nimB* genes. The transferability of the *cfiA*, *nimB*, and *tetQ* genes was tested by conjugation. The clonality of the isolates was tested by ERIC PCR (ca. 20 and 12 *cfiA*-negative, and positive strains were additionally included respectively) and PFGE. In the case of one strain (*B. fragilis* O:21), the genomic sequence was determined by Next-Generation Sequencing (NGS).

Most of the isolates were resistant to piperacillin, piperacillin/tazobactam, cefoxitin, meropenem, metronidazole, clindamycin, and tetracycline and susceptible to moxifloxacin, and tigecycline. Whereas the *tetQ* gene was transferred to a susceptible host, the *cfiA* and *nimB* genes were not. Analysis of the excised circular intermediate of the tet^R elements indicated that they may be of different kinds. All the *nimB* genes and some of the *cfiA* genes were preceded by IS*1186,* and this latter held true for the *ermF* IS*4351* pair. A ca. 4 kb portion nucleotide sequence surrounding the *cfiA* gene in *B. fragilis* 1672 was determined by long PCR amplification and the structure of this region was found to be similar in the other strains. ERIC PCR typing implicated a clonal relationship of these isolates, while the PFGE typing gave a diverse pattern and suggested some evolutionary history. In the genome-sequenced strain, we found *tetX* and *tetX1* in the constitution as in Tn*4351*, but without the IS*4351* pair which implied that this strain served as a source for emerging Tn*4351* by the skipping of IS*4351* elements upstream and downstream of *tetX* and *tetX1*, recently found for the *cfiA* gene and IS*613* in the pBFUK1 plasmid. However, in the genome sequence of our strain the *cfiA* and *nimB* regions were not fully clarified, possibly because the structure is difficult to resolve by NGS.

The findings above conclude that this clone emerged early and served as a reservoir for resistance elements and multiresistant *B. fragilis* strains worldwide, at least before the widespread occurrence of tet^R elements among *B. fragilis* strains.

EVALUATION OF THE ANTIMICROBIAL SUSCEPTIBILITY PROFILING OF TIGECYCLINE AND OTHER ANTIBIOTICS AGAINST CLINICAL ISOLATES

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During 2012-2013, 579 strains of various pathogens were isolated from clinical samples in a survey of their antibiotic susceptibility against a number of synthetic antibiotics. Isolated pathogens were belonging to the following genus: *Enterobacteriaceae* (39.7%), *Staphylococcus* (35.1%), other non-*Enterobacteriaceae* (10%), *Enterococcus* (7.4%), *Streptococcus* other than *S. pneumoniae* (2.6%), *Haemophilus* (2.2%), *S. pneumoniae* (1.7%), *Moraxellaceae* (0.9%) and other Gram positives (0.3%). Percentage of Gram positives was 52.8% and Gram negatives 47.2%. Pathogens were isolated from clinical patients (males 52.5%, females 47.2%) categorized in age groups as follows: below 20 years (6.6%), 21-39 years (33%), 40-59 (33.2%), 60-79 (22.3%) and over 80 years of age (5%). Most common strain was *Staphylococcus aureus* MSSA (14%) followed by *E. coli* (13.3%), *Klebsiella pneuomoniae* (11.4%) and *S. aureus* MRSA (11.1%).

Identification of the organisms was performed *via* Vitek2 (Biomerieux) and susceptibility by using the micro-dilution method in order to estimate Minimum Inhibitory Concentration (MIC). All isolates were categorized as susceptible (S), intermediate (I) or resistant (R) according to the guidelines set by CLSI.

Test battery of antibiotics consisted of Amikacin, Amoxicillin/Clavulanic acid, Ampicillin, Ampicillin /Sulb, Aztreonam, Cefepime, Ceftazidime, Ceftriaxone, Clindamycin, Colisitin, Daptomycin, Doripenem, Ertapenem, Erythromycin, Oxacillin, Penicillin, Piperacillin/Tazobactam, Quinupristin/Dalfopristin, Teicoplanin, Tigecycline, Vancomycin. High-level aminoglycoside resistance was assessed by growth at high concentrations of Gentamicin (500 μg/mL) and Streptomycin (1000 μg/mL). Finally, strains were also tested phenotypically for ESBL.

The results showed that noticeable resistance was observed for: *Acinetobacter baumannii* in amikacin (83.3%), *E. faecium, E. coli, Klebsiella spp* in ampicillin (88 – 90.5%), *Proteus spp* in ampicillin (100%), *E. coli* and *Kl. pneumoniae* ESBL in aztreonam (100 and 84.6%), *E. coli* ESBL in cefepime (100%), *Acinetobacter baumannii* (83.3%) and *Kl. pneumoniae* (84.6%) in ceftazidime, *Kl. pneumoniae* in ertapenem (84.6%), *E. faecium* in erythromycin (100%), *A. baumannii* in imipenem and meropenem (100%), *E. faecalis* (88.9%) and *E. faecium* (100%) in levofloxacin, *S. aureus* in oxacillin (100%), *Proteus mirabilis* in tigecycline (100%) and *E. faecalis in* quinupristin/dalfopristin (100%). In general, most of our strains proven susceptible to the antibiotics tested and tigecycline exhibited a good performance against the bacteria tested.

730 ***SATELLITE SYMPOSIUM II:*** **PRIMARY PREVENTION OF *CLOSTRIDIUM DIFFICILE* INFECTION WITH SPECIFIC PROBIOTICS**

META-ANALYSES OF PROBIOTIC PRIMARY CDI PREVENTION STUDIES

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It has become increasing apparent that infection control strategies used to interrupt horizontal transmission of *C. difficile* are unlikely to eliminate all transmission of this pathogen in the healthcare setting. An alternate strategy is to reduce the risk of infection in those exposed to *C. difficile*. Probiotics for secondary prevention of CDI have been disappointing and have generated skepticism for any role of probiotics in preventing CDI. However new understanding of the host colonic microbial diversity and the gradation of flora disruption in patients at risk for CDI to those with recurrent CDI may suggest a role for probiotics in primary CDI prevention.

Two meta-analyses of probiotics for prevention of primary CDI have recently been reported. The first meta-analysis included randomized, placebo-controlled efficacy studies of probiotic use among adults receiving antibiotics, in which CDI was one of the outcomes measured. Only probiotics that were included in more than one randomized trial were studied. In this analysis, three studies that used the probiotic combination *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* LBC80R and a combined analysis of those studies with four studies that used *Saccharomyces boulardii*, showed lower CDI rates in recipients of probiotics compared with recipients of placebo (risk ratio = 0.39; 95% confidence interval 0.19–0.79). The second meta-analysis included randomized studies of adults and children receiving antibiotics that compared any strain or dose of a specified probiotic with placebo or with no treatment control and which reported the incidence of CDAD (i.e., CDI). This analysis reported that probiotics reduced the incidence of CDAD by 66% (Pooled RR, 0.34 [95%CI, 0.24 to 0.49]; I2= 0%). In addition, 9.3% of probiotic recipients experienced adverse events compared to 12.6% of controls (RR, 0.82 [CI, 0.65 to 1.05]; I2= 17%).

While potential flaws in study design were identified, a review of the available literature suggests that the primary prevention of CDI with specific probiotic agents may be achievable. Additional studies of sufficient size and with rigorous design are needed to confirm these findings.

IMPACT OF UNIVERSAL PROBIOTIC ADMINISTRATION TO ANTIBIOTIC RECIPIENTS IN A COMMUNITY HOSPITAL

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Background: In 2003, hospitals in Quebec, Canada experienced an increase of NAP1/027 *Clostridium difficile* infections following antibiotic administration (CDIAA). At Pierre-Le Gardeur Hospital (PLGH), the incidence increased from 10 to over 25 cases per 1000 patient admissions.

Methods: We reported a quasi-experimental, prospective cohort study evaluating the effect on CDIAA of a probiotic added to existing *C. difficile* infection (CDI) standard preventative measures (SPM) in 31,832 hospitalized patients receiving antibiotics. Phase I (1580) measured the impact of SPM alone. In Phase II, 50 to $60x10^9$ cfu daily dose of oral *Lactobacillus acidophilus* CL1285 and *L. casei* LBC80R probiotic formula (Bio-K+) was administered to all patients receiving antibiotics. Phase III included the same intervention after a move to a new hospital facility. Phases II and III included 4968 patients. During Phase IV, 25,284 patients were submitted to the same regimen but outcome data were compared to those of similar hospitals in Quebec.

Results: At the end of Phase III, CDIAA had decreased from more than 18 cases per 1000 patient admissions in Phase I to less than 5 cases. Reductions of CDI cases (73%) ($p<0.001$) and severe CDI cases (76.4%) ($p<0.001$) were observed. CDI recurrence rate was reduced by 39% ($p<0.001$). During the following 6 years, the CDI rate averaged 2.71 cases per 10,000 patient-days at PLGH compared to 8.50 cases per 10,000 patient-days in equivalent hospitals located in Quebec.

Study limitation: This study was not a randomized clinical trial but was an open, prospective study. Also, following Phase II, PLGH moved into a new facility and this could have contributed to lower CDI rates.

Conclusions: A specific probiotic product added to SPM and antibiotic stewardship activities resulted in a further reduction in CDI rates and was shown to be safe.

845 *SESSION XII:* VAGINAL MICROBIOME

THE VAGINAL MICROBIOME: WHAT'S NEW SINCE 2012?

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In the last decade, cultivation-independent approaches have expanded the known bacterial communities associated with the human reproductive tract, in particular, the vaginal microbiome. Despite the fact that hundreds of "new" species have been associated with the dysbiosis known as bacterial vaginosis (BV), these efforts have not yet answered some of the key riddles that characterize this environment. For one, even when sensitive and comprehensive assays are used, the environment we define as normal—one that has been epidemiologically associated with favorable reproductive health outcomes—still appears to be somewhat monolithic, dominated by lactobacilli specific to the human vagina that maintain characteristically low pH (<4.7) (namely, *L. crispatus* and *L. jensenii*). Second, although the dynamic nature of this microbiome has been confirmed, with daily sampling revealing marked changes in bacterial concentrations often related to sex, menses, or other external factors that better depict the disruption in the ratio of key lactobacilli to commensal anaerobes that results in BV, we still do not understand the precipitants or pathogenesis of this disruption, or even the sequence of two key events (loss of desirable lactobacilli, or establishment of BV-associated bacterial dominance). Third, the apparently essential role of *Gardnerella vaginalis,* despite its lack of specificity for the condition of BV, continues to be apparent; in particular, its role in the biofilm associated with BV appears to be fundamental to the process. Fourth, time-honored means of diagnosing BV—particularly the Nugent score for Gram staining—need to be-reevaluated in light of the new techniques to visualize recently described bacteria that may be pivotal to the numerical score, and may be associated with distinct clinical and pathologic endpoints. Fifth, the role of sex hormones (estrogen and progesterone), especially when delivered as contraceptives, requires elucidation. Finally, advances in defining the microbiologic communities that characterize BV have not yet been translated into more sophisticated, or successful, approaches to treating it, or to facilitating maintenance of a healthy vaginal environment. This session will provide recent developments in our understanding of the epidemiologic and microbiologic data that inform understanding of this complex dysbiosis.

WHICH MEMBERS OF THE VAGINAL MICROBIOME ARE ASSOCIATED WITH AMNIOTIC FLUID INFECTIONS AND PID?

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Microorganisms colonizing the vagina can ascend to cause infections of the upper genital tract including amniotic fluid infection (AFI) and pelvic inflammatory disease (PID). Microbial invasion of the amniotic fluid accompanied by inflammation increases the risk of rapid preterm delivery, while microbial colonization without inflammation has limited sequelae. The microbes detected most often in women having AFI with inflammation include *Fusobacterium nucleatum, Sneathia sanguinegens, Bacteroides ureolyticus, Gardnerella vaginalis,* Group B streptococci, *Haemophilus influenzae,* and *Leptotrichia amnii.* Although *U urealyticum* is a frequent isolate, it is most often present without inflammation and is not associated with rapid preterm birth. During PID, microorganisms can ascend to cause inflammation of the endometrium and fallopian tubes which can lead with infertility. In contrast to PID in which 25% of women have endometrial infection due to *Chlamydia trachomatis* or *Neisseria gonorrhoeae,* these STIs rarely invade the amniotic fluid. Salpingitis and endometrial inflammation are predictors of reduced fertility. However, 25% of salpingitis and half of endometritis is caused by non-STI microorganisms including *Leptotrichia*, *Atopobium vaginae*, clade B *Gardnerella vaginalis, Prevotella spp, Peptostreptococcus* and *Haemophius influenzae.* When direct sequencing and cultivation are applied to endometrial tissues, those tissues which are positive by both direct sequencing and cultivation are the most likely to have inflammation, whereas those samples which are positive only by sequencing or culture are less likely to have evidence of inflammation. Comparing direct sequencing and cultivation methods for women with amniotic fluid infections and PID, *Sneathia* and *Leptotrichia* are detected entirely by sequencing methods, whereas *Fusobacterium, Prevotella, Peptostreptococcus* and *Bacteroides* are detected using both cultivation and sequencing, and *Ureaplasma* is detected more frequently by culture. Both amniotic fluid infections and PID are characterized by a diverse range of anaerobic bacteria, some of which can be detected only through direct sequencing methods. Although a broad range of microorganisms can be recovered from the upper genital tract of women with AFI or PID, only a subset of these cause inflammation and adverse reproductive sequelae.

THE DIFFERENTIAL ROLES OF VAGINAL BACTERIA IN TUNING MUCOSAL IMMUNITY

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The mechanisms underlying the wide spectrum of clinical manifestations of bacterial vaginosis (BV)—ranging from lack of symptoms and inflammatory cell efflux to severe discharge, pain, and irritation—are poorly understood. Our goal is to elucidate the role of most common microbiota constituents in controlling or evading the vaginal immune environment that defines clinical diversity. We have established an experimental model of bacterial colonization utilizing human vaginal and cervical epithelial cells in combination with peripheral blood monocytes (PBMC), resident bacteria, pathogens and synthetic analogs of microbe-associated molecular patterns (MAMPs) to mimic the environment during healthy and inflamed conditions. In this model bacterial viability and colonization patterns are assessed by colony forming units, gene expression—by nuclease protection assay, NF-κB activation—by a luciferase reporter, and protein levels and phosphorylation—by Meso Scale Discovery multiplex and western blot. We found that the epithelial cells and the PBMC, exposed to the colonized vaginal environment, differ significantly in their responses to vaginal lactobacilli and BV-bacteria. The PBMC mounted a strong IL-1, IL-8, IL-10, IL-13, TNFα and IFNγ response to all bacteria, including lactobacilli; in contrast, the epithelial cells, despite NF-κB nuclear translocation in response to colonization, were immunotolerant to lactobacilli and expressed proinflammatory proteins most vigorously in response to *A. vaginae* and *G. vaginalis*. *P. bivia* inhibited proinflammatory chemokine responses to MAMPs and infection, and inhibited immunoregulatory chemokines in both the epithelia cells and PBMC. These data suggest that by controlling the type of bacteria predominating in the vaginal microbiota and colonizing the vaginal epithelium, we may be able to control the efflux and activation of immuno-inflammatory cells and the productive immune responses to invasive pathogens.

EFFECT OF INTRAUTERINE CONTRACEPTION ON VAGINAL MICROBIOTA

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The vaginal microbiota has the potential to influence susceptibility to sexually transmitted infections. Therefore, it is critical to determine if particular contraceptive methods alter the vaginal microbiota and, thus, potentially alter susceptibility to infection. The purpose of this study was to determine if the use of a copper intrauterine device (Cu-IUD) or a levonorgestrel intrauterine system (LNG-IUS) changes the vaginal microbiota. Vaginal swab samples were obtained from 100 women during their first year of Cu-IUD (50 women) or LNG-IUS (50 women) use at baseline, 6 months and 12 months through the Contraceptive CHOICE Project. DNA was isolated from the swabs and portions of the bacterial 16S rRNA gene were amplified and sequenced by 454 (V3-V5) and Illumina (V4). The sequences were used to compare bacterial communities with the software package mothur. Our preliminary data analysis indicates that the vaginal microbiota did not change significantly with the use of either type of intrauterine contraception and is therefore unlikely to contribute to altered infection susceptibility.

1015 ***SESSION XIII: CLOSTRIDIUM DIFFICILE EPIDEMIOLOGY & PREVENTION***

UPDATE ON THE EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE* INFECTIONS FROM THE CDC

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U.S. hospital discharges coded with *Clostridium difficile* infections (CDI) plateaued at historic highs from 2008-10 and then continued their increase in 2011 with projected further increases through 2013. These are the latest in a string of increases that began back in 2000, coinciding with the emergence of NAP1. Since that emergence, and as the full scope of this epidemic has become apparent, new U.S. surveillance systems have been developed. The Emerging Infections Program (EIP) has described how only 23% of CDI cases have their onset in hospitals, while 26% onset in nursing homes, 19% in the community among recently discharged patients, 26% in the community among patients with recent outpatient visits only, and 6% in the community among patients with no recent healthcare exposures. Among the latter two categories combined, 36% have had no recent antibiotic exposures; 31% of antibiotic non-exposed patients received proton pump inhibitors. Patients with CDI and no or minimal healthcare exposures are more likely to have been exposed to infants or household members with CDI. Onset of CDI in the community following only outpatient healthcare exposures is even more common in children less than 18 years of age, among whom 71% fit this category. CDI incidence in children is highest among those 1 to 3 years of age. Although asymptomatic colonization in this age strata is common, similar disease severity across age groups suggests an etiologic role for *C. difficile* in these high rates. The NAP1 strain continues to be prevalent in the United States, accounting for 28% of infections with higher rates in healthcare vs. community cases. A recent analysis has confirmed results of several other studies suggesting the hypervirulence of NAP1: in a multivariate model including risk factors of age >65, non-white race, healthcare-associated epidemiologic class, a higher co-morbidity index, and antibiotic exposures in the previous 14 days, the NAP1 strain was an independent predictor of severe disease and outcomes including 14-day mortality. Meanwhile, over 4,000 acute care hospitals are now reporting hospital-onset CDI LabID events via CDCs National Healthcare Safety Network to fulfill the Centers for Medicare Services incentive for public reporting. A risk adjustment model that includes the prevalence of CDI on admission and the type of laboratory test (higher rates are associated with use of nucleic acid amplification tests) has been developed to report a standardized infection ratio (SIR). In 2012, there was a 2% decrease in the national SIR compared to a 2011 baseline.

NEW INSIGHTS INTO *CLOSTRIDIUM DIFFICILE* TRANSMISSION IN THE HOSPITAL BASED ON WHOLE GENOME SEQUENCING

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Whole genome sequencing (WGS) has the potential to revolutionalise our understanding of the epidemiology of *C. difficile* infection (CDI). A recent UK study using a cohort of over 1200 CDI cases has highlighted the value of WGS to understand *C. difficile* transmission (outside of the epidemic setting). The majority (~65%) of CDI cases could not be linked, using highly discriminatory WGS, to previous cases. This assumes that there is a low rate of genetic mutation in *C. difficile* strains; in 145 patients we found that up to 2 single-nucleotide variants (SNVs) are seen in isolates from patients who provided multiple faecal samples up to 124 days. 45% of CDI cases had isolates with >10 SNVs from all previous cases. The sources for transmission in CDI cases (n=120) that had isolates <2 SNVs apart, but who had no hospital or community contact with another patient, remain unclear. Reductions in CDI incidence over time were similar among linked and unlinked CDI cases, suggesting that there is a common effective intervention; altered antimicrobial prescribing may be this common intervention. Distinct subtypes continued to be identified throughout the study, which suggests that there is a considerable reservoir(s) of *C. difficile*. Thus, *C. difficile* may be a wider public health issue than previously thought.

DEVELOPMENT AND PROGRESSION OF A CANDIDATE *CLOSTRIDIUM DIFFICILE* VACCINE FOR THE PREVENTION OF SYMPTOMATIC CDI

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Clostridium difficile (C. diff) is a potentially life-threatening, spore-forming bacterium that causes intestinal disease. The risk of contracting a C. diff infection (CDI) increases with age, antibiotic treatment and time spent in hospitals or nursing homes, where multiple cases can lead to outbreaks. Despite improvements in reducing some health care-associated infections, CDI remains at historically high levels. Additionally, there is recent evidence that a significant number of C. diff infections occur in the community, and among people who have not been in recent or prolonged contact with health care facilities.

Sanofi Pasteur's candidate vaccine is designed to induce an immune response to neutralize and prevent the damaging effects of the C. diff toxins in the human gut. Like other toxoid vaccines (e.g., tetanus and diphtheria), the C. diff candidate vaccine is composed of inactivated native toxins; and as such, is expected to elicit broader immune responses than recombinant truncated-toxin antigens. In pre-clinical animal studies, Sanofi Pasteur's C. diff candidate vaccine elicits anti-toxin A and B IgG antibodies (as measured by Enzyme Linked Immunosorbent Assay) as well as anti-toxin A and B neutralizing antibodies (as measured by Toxin Neutralization Assay). In addition, protective, dose-dependent, toxoid-vaccine induced immune responses have been demonstrated in both the active and passive hamster challenge models.

The industrialization and safety profile of toxoid-based vaccines have been proven over many years. The toxoid-vaccine's large-scale manufacturing process is accompanied with established and validated analytical testing procedures. Bridging of key quality attributes between large-scale and small-scale toxoid-vaccine lots has been verified through bio-comparability studies measuring physicochemical, biochemical and immunological parameters of the vaccine lots.

The C. diff candidate vaccine has progressed through Phase I and II safety and immunogenicity studies. Currently, the industrial-scale C. diff toxoid-vaccine is being studied for the prevention of primary disease caused by CDI in a randomized, observer-blind, placebo-controlled, multi-center, multi-national Phase III trial called *Cdiffense*. The U.S. Food and Drug Administration (FDA) granted fast-track designation to Sanofi Pasteur's investigational C. diff vaccine in 2010. The challenges and solutions for developing such a novel vaccine will be discussed.

*On behalf of Sanofi Pasteur's *C. difficile* Toxoid Vaccine Global Development Team

LABORATORY-BASED SURVEILLANCE OF *CLOSTRIDIUM DIFFICILE* STRAINS CIRCULATING IN AUSTRALIAN HEALTHCARE

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Since 2011 Australia has seen a significant rise in the rate of *C. difficile* infection (CDI) that cannot be explained by improved diagnostics and increased awareness. To investigate this issue, we conducted laboratory-based surveillance of the molecular types of *C. difficile* circulating in the Australian healthcare setting. A collection of 556 *C. difficile* isolates from hospital-identified cases of CDI was compiled from participating hospitals and laboratories across all Australian States over a 1 month period spanning October and November 2012. Isolates were typed by PCR ribotyping. Basic demographic and clinical data were collected for a subset of cases. The 10 most common ribotypes comprised 64.7% of the collection; the top three were 014/020 group (25.0%), 002 (10.3%) and 056 (6.1%). One isolate could not be ribotyped, while the remaining 109 isolates represented 65 ribotypes. Emerging ribotypes which were not seen previously in the top 10 or had increased in prevalence since a similar survey in 2010 were 056, 070 (4.1%), 054 (4.1%), 015 (3.8%), 017 (3.4%), 053 (2.9%) and 244 (2.5%). About half the cases were healthcare-acquired (HA, 50.5%) while 17.5% were community-acquired (CA). The median age of cases overall was 66 years, and this was significantly lower in CA cases (57 years) compared to HA cases (70 years, p=0.02). While no specific ribotypes were significantly associated with CA CDI in our survey, a number of ribotypes (056, 126, 127, 033) were also recently found in Australian production animals, indicating a possible community health threat in Australia. In conclusion, ribotypes 014/020 group and 002 remained the most common strains causing CDI in Australia. However, the molecular epidemiology of CDI in Australia appears to have changed considerably over the 2-year period since the last survey. Several ribotypes increased in prevalence significantly, notably 056, 017, 053 and 244. The reasons for this need investigating, including a search to confirm new reservoirs in production animals and food.

SXIII-5

DOES BINARY TOXIN CONTRIBUTE TO *CLOSTRIDIUM DIFFICILE* INFECTION?

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Clostridium difficile infection (CDI) is the main cause of healthcare acquired diarrhoea in the developed world where it imposes a significant financial burden and is implicated in higher mortality rates than MRSA. Toxin A and B are the two main virulence factors produced by *C. difficile*. However most epidemic strains (*e*.g., PCR-Ribotype 027 (027/NAP1/B1) and 078) that have emerged in recent years and are held responsible for more severe disease, higher relapse rates and mortality produce in addition a binary toxin (*Clostridium difficile* toxin (CDT)).

The importance of CDT in disease has not yet been fully elucidated. Early data revealed similarities to *Clostridium perfringens* iota toxin confirming ADP-ribosyltransferase activity capable of covalently modifying cell-actin. Conversely, no role could be suggested in disease as no cytotoxicity was measured on cells and no lethal effect in mice observed upon injection of purified CDT [Popoff *et al*, 1988]. However, a recent study found CDT protein levels to be much higher when measured *in situ* than when tested *in vitro* [Carman *et al*, 2011]. Furthermore, it was shown *in vitro* that purified CDT confers increased adherence to epithelial cells through the formation of protrusions [Schwan *et al*, 2009].

Given the still unknown but possibly important role of CDT in disease, we created a series of stable, isogenic toxin A, B and CDT mutants in the UK outbreak strain *C. difficile* R20291 (027/NAP1/B1) using the ClosTron. Strains were characterised *in vitro* through cytotoxicity experiments and adherence to Caco2 cells. They were then compared *in vivo* in the mouse colonisation and the hamster virulence model. Part of these data have recently been published [Kuehne *et al*, 2014], showing like in our first study [Kuehne *et al*, 2010] that each of the two main toxins A and B alone can cause fulminant disease *in vivo* and have produced tantalising data that CDT may also be contributing to disease. These data and unpublished results form adherence and colonisation experiments will be discussed.

1345 ***SESSION XIV: CLOSTRIDIUM DIFFICILE TREATMENT & IMMUNITY***

OPTIMIZING EXISTING THERAPIES FOR *CLOSTRIDIUM DIFFICILE* INFECTIONS

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Until recently two antimicrobial agents, metronidazole and vancomycin, have been the mainstays of treatment for *C. difficile* infection (CDI). Current SHEA/IDSA guidelines recommend metronidazole for treatment of mild-to-moderate infections and vancomycin for severe CDI. Since publication of the guidelines, new data have emerged showing decreased response rates to metronidazole and the approval of a new agent, fidaxomicin. Fidaxomicin has been shown non-inferior to vancomycin for intial treatment response and superior to vancomycin for sustained response 25 days after treatment. For reasons based primarily on cost, fidaxomicin has been reserved in many centers for patients who have failed or had CDI recurrences following metronidazole and vancomycin treatment. Given the profound and progressive disturbance of normal colonic microbiota in patients with multiple recurrent CDI, standard 10- to 14- day treatment courses with any of these agents may not give the same cure rates seen as in patients with a first episode of CDI. Alternate treatment strategies, including tapering and pulsing the dose after a treatment course and post-treatment 'chaser' regimens using an agent with less microbiota-disruptive effects may improve outcomes in these patients. Other available, but not FDA-approved antibacterial agents for CDI, include nitazoxanide, rifaximin, and bacitracin but need further study to determine their role in the management of CDI. Additional, well-designed clinical trials are needed to further optimize treatment strategies using existing antimicrobial therapies, particularly in patients with recurrent CDI.

EMERGING DRUGS AND VACCINES AGAINST *CLOSTRIDIUM DIFFICILE:* A NEW STRATEGY FOR THE PREVENTION

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Since its discovery, treatment of *C. difficile* infection (CDI) has relied on two primary treatment antibiotics; either metronidazole or oral vancomycin. With the increased incidence and severity of CDI, interest in novel treatment strategies and new druggable targets for the treatment or prevention of CDI has increased. This session will focus on pre-clinical, early-, and late-stage drug development for novel therapies directed against *C. difficile*. Novel treatment strategies with existing therapies including anti-inflammatory and flora-restoration agents will be highlighted. Other druggable targets directed against *C. difficile* will be explored with an update on drug or vaccine development against each target. Specific emphasis will be placed on emerging therapies in late-stage development. Finally, the potential role in therapy relative to existing therapies will be discussed.

The specific objectives of this session will be:

1) To highlight pre-clinical investigations on the role of anti-inflammatories in the treatment of CDI
2) To review early- and late-stage pharmacologic agents for treatment or prevention of CDI
3) To understand the potential role of new agents to optimize the treatment of CDI

A NEW STRATEGY FOR THE PREVENTION OF *CLOSTRIDIUM DIFFICILE* INFECTIONS

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We have targeted spore germination to prevent *Clostridium difficile* infection (CDI). CDI is a leading cause of antibiotic-associated diarrhea, a major nosocomial complication. The infective form of *C. difficile* is the dormant and resistant spore. We recently reported that bile salt analogs can inhibit *C. difficile* spore germination *in vitro*. In this study, we tested different bile salts for efficacy in preventing CDI. We found that CamSA, a *m*-aminobenzene sulfonic acid bile salt derivative, was sufficient to prevent CDI in mice without any observable toxicity. We also showed synergistic protection between CamSA and vancomycin in the CDI hamster model.

We further characterized CamSa's *in vitro* stability, distribution, and cytotoxicity. CamSA is stable to simulated gastrointestinal (GI) environments, but will be slowly degraded by members of the natural microbiota. Our data also suggests that CamSA will not be systemically available, but instead will be localized to the GI tract. CamSA shows no toxic effects towards vegetative bacteria, or mammalian cells.

Several experiments support a mechanism whereby the anti-germination effect of CamSA is responsible for preventing CDI signs. Lower CamSA doses resulted in delayed CDI onset and less severe signs of disease. By varying the timing of CamSA dosage, we estimated that *C. difficile* spores germinated and established infection less than 10 hours after ingestion. We also showed that ingested *C. difficile* spores rapidly transited through the GI tract and accumulated in the colon and cecum of CamSA-treated mice. From there, *C. difficile* spores were slowly shed over a 96-hour period and were quantitatively recovered from feces and intestinal content.

To our knowledge, this is the first report of using molecular probes to both prevent CDI and obtain disease progression information for *C. difficile* infection. This approach represents a new paradigm in CDI treatment. Instead of compromising the microbiota of CDI patients with strong antibiotics, anti-germination therapy could serve as a microbiota surrogate to curtail *C. difficile* colonization of antibiotic-treated patients.

GLOBAL ANALYSIS OF THE ROLE OF INFLAMMATION IN *CLOSTRIDIUM DIFFICILE* COLONIZATION AND DISEASE

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The factors that control the outcome of *C. difficile* colonization in the large intestine (epithelium destruction/inflammation vs. persistence without damage) are multi-factoral and include both microbiome-derived factors as well as host-derived signals. We have been investigating the contribution of host-derived signals to epithelial damage, inflammatory cell influx and *C. difficile* levels using a murine model of acute infection by two different *C. difficile* strains, VPI10463 and 630, that have markedly different disease kinetics. We have used variety of broad-spectrum antibiotics in conventional mice, as well as germ-free mice, and have altered the host through genetic single gene knockouts (IL-23, IL-17, Reg3γ) and treatment with neutralizing or depleting antibodies against various signals or cells (GM-CSF, TNFα, IL-22, CCR2, CD160, Gr1). The host response has been followed by flow cytometry, histology and qPCR (90 gene panel). A significant influx of activated neutrophils and a number of different types of macrophage subsets characterize the host response. It involves an IL-23/IL-22/CD160/Reg3γ signaling pathway, STAT3 phosphorylation, induction of a number of CC and CXC chemokines, modulation of anti-microbial peptide expression, induction of *Slpi* expression in the epithelium, and, surprisingly, an anti-inflammatory role for TNFα signaling in modulating IL-1β induction and macrophage influx. Gr1+ cells (neutrophils and a subset of macrophages) play little role in modulating this signaling network. We have identified that there is a well-defined cascade of events that accompany the development of the inflammatory response, regardless of the broad-spectrum antibiotic used or even in the complete lack of a microbiome, and these are induced to a greater or lesser degree in concert with the level of disease severity elicited by the different strains of *C. difficile*. Interestingly, modalities that reduced or exacerbated inflammation could do so without affecting the level of *C. difficile* colonization, implicating a dichotomy between factors that promote colonization and those that promote inflammation.

Support: NIH grant U19AI090871

1510 SESSION XV: BACTERIAL THERAPY

NON-TOXIGENIC *CLOSTRIDIUM DIFFICILE* (NTCD) FOR PREVENTION OF RECURRENT *C. DIFFICILE* INFECTION (CDI)

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In Phase 1, VP20621, NTCD strain M3, was administered to volunteers following antibiotic treatment and was well tolerated and led to high rates of NTCD colonization in stool. A Phase 2 study evaluated VP20621, when administered to patients following antibiotic treatment for CDI. Adult subjects who had recovered from CDI and completed a course of metronidazole or oral vancomycin were randomized to receive once daily placebo or VP20621 spores (104 x 7days, 107 x 7days or 107 x 14days), beginning 1-2 days after the last day of antibiotic. *C. difficile* stool cultures (with testing of isolates for toxin by EIA) were performed at baseline (NTCD Day 1) and Weeks 1, 2, 3 and 6. Subjects were monitored for recurrent CDI (defined as ≥3 unformed stools within 24 hours, positive *C. difficile* stool assay and no other likely cause of diarrhea). All culture-positive patients were followed for 6 months or until no longer colonized.

168 subjects were randomized and dosed: overall median age 59 years; 39% ≥65 years old; 62% female. There were no notable safety differences between the VP20621 dose groups. Mild-moderate headache was the only notable associated adverse event (2% placebo, 10% VP20621). Treatment-emergent serious adverse events: 7% placebo, 3% VP20621; none were related to study drug. Data in table are through study Week 6.

	Placebo	VP20621			
		10^4 x 7d	10^7 x 7d	10^7 x 14d	All VP20621 doses
Number of patients	43	41	43	41	125
Percent NTCD detected in stool during administration	0%	54%	79%	73%	72%
p value		<0.0001	<0.0001	<0.0001	<0.0001
CDI Recurrence rate	30%	15%	5%	15%	11%
p value		0.11	0.002	0.10	0.003

VP20621 was well tolerated at and reduced the incidence of CDI recurrence by ≥50% vs. placebo for all doses, with a similar reduction in need for antibacterial treatment for CDI. Dosing at 10^7 spores/day for 7 days appears to be sufficient for effective colonization. All subjects lost colonization with VP20621 by 22 weeks. VP20621 is a novel biotherapeutic approach to prevention of CDI recurrence (and potentially primary CDI as well) which warrants further development.

TREATMENT APPROACHES FOR RESOLVING RECURRENT *CLOSTRIDIUM DIFFICILE* INFECTIONS

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The incidence of recurrent *Clostridium difficile* infection (RCDI) has increased dramatically during the last decade, but consistent treatment guidelines do not exist in published literature. Successful therapy depends on the elimination of *C. difficile* vegetative cells and the spore form reservoir. Bacteriotherapy aims at restoring bacterial richness and diversity in the lower intestinal tract through the introduction of nonpathogenic bacteria into the colon, and has so far been the most successful treatment modality for resolving relapsing diarrhea caused by RCDI. Treatment strategies have included the use of probiotic agents, "synthetic stool substitute," or instilling a suspension of fecal matter collected from a healthy donor (fecal microbiota transplantation or FMT) into the GI tract, and success rates have averaged between 80-100%. Probiotic agents administered alone have had only limited success. This presentation will discuss some of the technical aspects of FMT, review the process recommended to identify appropriate patient and donor candidates, outline potential expected and unexpected adverse effects, and review the global experience with FMT for the treatment of RCDI. Some patients elect not to be treated with FMT, and may benefit from treatment using a staggered intermittent vancomycin withdrawal regimen combined with daily ingestion of kefir (a probiotic readily available in food stores) as an alternative to FMT.

1610 *SESSION XVI:* NEW INSIGHTS INTO *CLOSTRIDIUM DIFFICILE* PATHOGENESIS

IN-DEPTH PROTEOMIC ANALYSIS OF THE TOXIGENIC AND NON-TOXIGENIC *CLOSTRIDIUM DIFFICILE* SECRETOME

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The main *Clostridium difficile* (Cdiff) virulence factors are two toxins (TcdA and TcdB) that are released in the late log and stationary growth phases. Other secreted proteins may play important roles in Cdiff pathogenesis. In addition, non-toxigenic Cdiff strains exist and can prevent colonization by toxigenic strains. In this study, we used high-throughput proteomics to characterize, quantify, and compare the secretome of toxigenic and non-toxigenic Cdiff clinical isolates. An array of complex technologies that incorporates protein separation methods, liquid chromatography-mass spectrometry (LC-MS), and bioinformatics was used to analyze *in vitro* secretomes of toxin-producers Cdiff clinical isolates 027, 078, 014, and non-toxigenic Cdiff strains in exponential and stationary growth phases. The complexity of the protein suspensions was decreased by applying different fractionation methods. Briefly, fractions were digested with trypsin and the resulting peptides were analyzed by LC-MS and Tandem-MS. Matched peptides were validated using Scaffold. The next phase of the study entailed in-depth analysis of the most abundant proteins.

Both qualitative and quantitative differences in proteins associated with Cdiff virulence were observed. Moreover, we uncovered the *in vitro* toxigenic and non-toxigenic Cdiff secretome repertoire. Over two hundred proteins were identified with high confidence. The expected toxins, TcdA and TcdB, as well as binary toxin were verified as expected. In addition, key virulence factors including adhesins and enzymes that are rare or non-existent in the non-toxigenic strains secretome were detected to a higher and variable extent in the toxigenic isolates. Further in-depth analysis of the most abundant proteins, including toxins (TcdA, TcdB, binary toxin) and virulence factors (S-layer proteins, adhesins, capsule, flagelin, and CodY) revealed posttranslational modifications and additional peptide sequence information suitable for further investigations. Our results provide novel insights into the complexity of toxigenic and non-toxigenic Cdiff secretome. These data highlight the importance of the secretome as a potential reservoir of protein biomarkers and diagnostic targets that may offer new information on Cdiff pathogenesis and diagnosis.

DIFFERENT WAYS TO DIE: EPIDEMIC-ASSOCIATED *CLOSTRIDIUM DIFFICILE* REMODELS ITS CELL-SURFACE, AND MANIPULATES THE HOST INNATE IMMUNE SYSTEM TO CAUSE SEVERE DISEASE

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Background and rationale: *Clostridium difficile* infection (CDI) is a predominantly antibiotic-associated diarrhea caused by the anaerobic bacterium *C. difficile* (CD). In the past 14 years, CD strains of increased virulence and persistence have been recovered from human and veterinary CDI; however, no new pathogenic factors have been identified. We and others have shown that these newer epidemic-associated strains have generally similar intoxication and sporulation characteristics when compared to their older (historic), phylogenetically-related counterparts. Therefore, our hypothesis is that the increased virulence of epidemic-associated CD is due to altered bacterial colonization that results in both persistent and severe disease.

Methods and results: We used comparative proteomics, antisense-RNA knockdown studies, genetic analyses, and *in vivo* approaches, and determined that epidemic-associated CD strains exhibit a remodeled cell-surface with profoundly altered host innate-immune evasion capabilities. Specifically, flagellar regulation, capsular polysaccharide display, and antimicrobial peptide resistance were all altered in epidemic-associated CD, and predicted to result in robust bacterial colonization *in vivo*. We constructed over 50 isogenic CD mutants in historic and contemporary isolates, targeting these cell-surface molecules. A subset of the mutants were tested in the hamster CDI model, and we found that altering CD surface protein display resulted in altered lethality, with corresponding changes in bacterial burden, intestinal inflammation, and intoxication.

Conclusions: Cell-surface remodeling in epidemic-associated CD is a dynamic, strain-specific process interwoven with the expression of CD toxins, and directly impacting disease establishment. Further, the specific CD surface proteins we have identified represent attractive vaccine or small-molecule targets for interventions aimed at abrogating CD establishment in the mammalian gut.

INTERSECTION OF METABOLISM AND PATHOGENESIS IN *CLOSTRIDIUM DIFFICILE*

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The ability to sense and adapt to nutrient availability is a universal trait in the bacterial world. For the human pathogen *Clostridium difficile*, this ability may be critical for its proliferation and the production of the two major toxins TcdA and TcdB during infection. Synthesis of the toxin proteins is regulated by several environmental factors, including the availability of certain carbon sources, fermentation products (butyrate) and amino acids (e.g., proline and the branched-chain amino acids (BCAAs) isoleucine, leucine and valine). Two global regulators, CodY and CcpA, are known to repress toxin gene transcription in response to the presence of the BCAAs and glucose, respectively. The role of proline can now be attributed at least in part to its role as an activator of PrdR. *C. difficile*, like many *Clostridium spp.*, uses the Stickland reactions (co-fermentation of pairs of amino acids) to generate ATP and NAD^+. One of the key Stickland enzymes, D-proline reductase (PR), which regenerates NAD^+, is positively regulated by PrdR. Moreover, proline and/or PrdR appeared to repress the transcription of additional NAD^+-generating pathways, such as the glycine reductase (GR), the butyrate production pathway and the succinate utilization pathway, suggesting a hierarchical control of NAD^+ regeneration. Here, we report evidence that modulation of the NAD^+/NADH ratio by PR allows Rex, a global redox-sensing regulator, to control succinate, butyrate and glycine reductase genes. The overlapping regulation of key metabolic pathways and toxin synthesis by CcpA, CodY, and PrdR/Rex suggests strongly that toxin production is a complex response by the bacteria to particular states of nutrient availability.

CONSERVED OLIGOPEPTIDE PERMEASES MODULATE SPORULATION INITIATION IN *CLOSTRIDIUM DIFFICILE*

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The Gram-positive, anaerobic organism *Clostridium difficile* is a gastrointestinal pathogen of humans and other animals and is the primary cause of antibiotic-associated diarrhea. To survive in oxygenic environments and be transmitted from host to host, *C. difficile* undergoes a complex morphological process to form a metabolically dormant spore. However, the regulatory processes by which *C. difficile* initiates and controls the early stages of sporulation are unknown. In *Bacillus subtilis*, sporulation is positively regulated by cell density through the uptake of small quorum sensing peptides (the Phr peptides). The Phr peptides are imported by the Opp (Spo0K) and App oligopeptide permeases. Orthologs of the Opp and App transporter systems are present in the *C. difficile* genome; however, a Phr ortholog is notably absent. Here, we investigated the role of Opp and App in regulating sporulation in *C. difficile*. In contrast to other spore-forming bacteria, we discovered that inactivating these permeases in *C. difficile* resulted in earlier and increased expression of early sporulation genes and a significant increase in the rate and frequency of sporulation compared to the wild-type strain *in vitro*. Disruption of either or both *opp* and *app* resulted in a hypervirulent phenotype as well as increased spores recovered from fecal samples in the hamster model of *C. difficile* infection. We observed that the increase in sporulation of the transporter mutants was associated with elevated expression of genes controlled by the global metabolic regulator, CcpA, suggesting that Opp and App indirectly inhibit sporulation through the activation of CcpA. Altogether, these results indicate that the Opp and App transporters serve a different function in regulating sporulation and virulence in *C. difficile* compared to other spore-forming bacteria and suggest that nutrient availability plays a significant role in pathogenesis and sporulation *in vivo*. This study is the first to link the nutritional status of the environment to sporulation initiation in *C. difficile*.

DEFINING THE EARLY STAGES OF *CLOSTRIDIUM DIFFICILE* SPORE GERMINATION

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C. difficile spores are the infectious form of the organism. Only the spore form can survive for extended periods of time outside a host in an aerobic environment. To cause disease, spores must germinate in the host to form the vegetative cells that are responsible for toxin production and disease. *C. difficile* spores germinate in response to certain bile acids and glycine (germinants). In *Bacillus subtilis*, the interaction of the spore germinant receptors with their respective germinants leads to the release from the core the large depot of Ca^{2+}-dipicolinic acid (DPA) resulting core rehydration. The release of DPA triggers the enzymatic degradation of the specialized peptidoglycan—cortex. Both of these processes occur enzymatically and lead to the loss of spore dormancy. *C. difficile* does not encode the canonical germinant receptors that are found in other spore-forming bacteria (e.g. *B. subtilis*). We recently identified the *C. difficile* spore bile acid germinant receptor as the germination-specific, protease-like, CspC protein. *C. difficile* CspC may be localized to the spore cortex or to the inner coat of *C. difficile* spores. We hypothesized that activation of CspC by a bile acid signal leads to the activation of the cortex hydrolase SleC. Thus, it would seem that in contrast to spores from other spore-forming bacteria, the interaction between *C. difficile* spores and spore germinants triggers the degradation of the spore cortex prior to the release of DPA from the core. Here, we analyze how germination occurs in *C. difficile* compared to *B. subtilis* by analyzing the kinetic release of both hexosamine and DPA during spore germination. The results from this study could have implications for other hypothesized *C. difficile* germinant receptors.

COMPLEX REGULATION OF *CLOSTRIDIUM DIFFICILE* BIOFILMS

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Clostridium difficile infection (CDI), a major healthcare-associated infection, occurs when the normal intestinal microflora is altered by treatment with antimicrobial agents. Recurrent clostridial infections and a rise of antibiotic resistant strains have complicated treatment of CDI. Although toxins are key virulence factors of *C. difficile*, factors affecting colonization of the gut are now known to be important during clostridial pathogenesis. Formation of adherent bacterial communities, as reported for other intestinal pathogens, may mediate persistence of *C. difficile* in the gut.

We demonstrated that *C. difficile* clinical strains, 630, and R20291, formed structured biofilms composed of proteins, DNA, and polysaccharide *in vitro* and that biofilm formation was a multifactorial process. A mutant in Spo0A, a master regulator of sporulation, was defective for biofilm formation, indicating a possible link between biofilms and spore formation. In recent work we have demonstrated that the anti-σ factor RsbW, which regulates the stress-induced alternative sigma factor σ^B is also involved in biofilm development. A *rsbW* mutant forms significantly more biofilm as compared to its wild type. Interestingly, this mutant is deficient in sporulation *in vitro*. We are currently investigating how these regulators mediate *C. difficile* biofilm development.

Our data suggest that complex regulatory networks that control alternate adaptive responses, including sporulation, govern *C. difficile* biofilm development.

SXVI-7

MECHANISMS OF IRON ACQUISITION IN *CLOSTRIDIUM DIFFICILE*

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Clostridium difficile is an important pathogen causing hundreds of thousands of infections per year globally. Research into mechanisms of pathogenesis of this organism has primarily focused on the major toxins. Iron acquisition mechanisms play an important role in the pathogenesis of many infectious microbes. Iron availability is limited in the host, requiring pathogens to develop specialized mechanisms for the acquisition of this important metal. To facilitate the identification of important iron acquisition mechanisms utilized by *Clostridium difficile*, we first performed microarray analysis to examine the global response of this pathogen to iron limited conditions. *C. difficile* 630 was grown in the presence or absence of the metal chelator, dipyridyl, which effectively induces iron starvation. RNA was isolated from cultures at 3, 5, and 7 hours and processed for hybridization onto a custom Agilent microarray. Using stringent statistical analyses, over 150 genes were identified as significantly induced by *C. difficile* during iron starvation. Candidate genes were then chosen from this list and were targeted for deletion using homologous recombination. These included genes annotated as ferric iron transporters, siderophore transporters, and others with potential roles in iron acquisition. Genes were deleted from *C. difficile* 630 and the resulting mutant strains were analyzed for growth under iron-starved conditions and for attenuation in a murine infection model. Understanding how *C. difficile* acquires iron in the host can lead to the identification of putative targets for future therapies.

1300 POSTER SESSION I: ECOLOGY OF THE MICROBIOTA

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Posters will be presented in Poster Session I
Sunday, June 29 1300-1400

FUSOBACTERIUM BACTEREMIA: RARE EVENT WITH CHANGING EPIDEMIOLOGY—DESCRIPTION OF 13 CASES

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Background: *Fusobacterium* (F) are gram (-) bacilli, obligate anaerobes which are part of the normal flora of oropharynx, GI and genital tracts. F bacteremia (FB) is uncommon, accounts for <1% of patients (pts) with bacteremia and carries a high mortality rate, up to 40.7%. We observed an increased number of FB cases and describe the changing epidemiology, characteristics and outcome of patients (pts) with FB at Carilion Clinic.

Methods: The number of pts with FB per year was obtained by a computerized database and retrospective chart analysis was performed. Demographic, laboratory, clinical, treatment and outcome data were collected. Statistical analysis was performed using Bivariate T–test.

Results: An increasing number of FB cases were observed with a significant trend noted over the past 7 years (p = 0.05). 13 unique patients with FB were identified. The mean age was 50.9 years with 53.8% males. The most common lab abnormalities were leukocytosis, elevated creatinine and low albumin.The source of FB was oropharyngeal in 5, GI in 2, Skin in 2 and unknown in 4 pts. *F. nucleatum* was the most common species isolated in 62% pts. 11 pts were treated with appropriate antibiotics. 2 pts who were not started on appropriate antibiotics died within 24 hours of FB. Mean length of hospital stay (LOS) was 18.3 days. 9 of 13 pts (69.2%) were admitted to ICU. 4 of 9 pts admitted to ICU had no prior medical problem. Overall, mortality was 30.7% (4/13 pts). Factors significantly associated with increased mortality included older age (p=0.00007), higher WBC count (p=0.042) and increased LOS (p=0.049).

Conclusion: FB is an uncommon event, with a significant increasing incidence noted at our institution. The most common source of infection was oropharyngeal. ICU admission was required in 62.9% of patients and overall mortality was high at 30.7%. Older age, higher WBC count and increased LOS were associated with a significant increase in mortality. Clinicians should consider including empiric anaerobic coverage in critically ill septic pts.

VIRULENCE FACTORS & IMMUNODIAGNOSIS OF *B.FRAGILIS* GROUP—AN EXPERIENCE OF TWO DECADES

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Purpose: To analyse the virulence factors and immunodiagnosis of *Bacteroides fragilis* group, a significant anaerobic human pathogen.

Methods & Results: This study was conducted in KMC Manipal & FMMC Mangalore for two decades, based on the investigations from patients with various clinical disorders attending these two hospitals.

Bacteriological characterization of 1992 anaerobes obtained from 3213 specimens, 896 (44.97%) were *B. fragilis* group, 849 were speciated biochemically, *B. fragilis* was 498 (58.66%) followed by *B. thetaiotamicron* 160 (18.85%). Virulence facors were demonstrated such as capsule, fimbriae, enzymes like β-lactamase, catalase, DNase, Neuraminidase, ability of *B. fragilis* to inhibit phagocytosis and intracellular killing of co-existing aerobe. Leucocyte Migration Inhibition experiments revealed that a soluble factor, probably succinic acid, present in the culture filtrate of *B. fragilis* at a low pH of 5.4, was capable of altering the leucocyte function and preventing its migration.

Serological study included 585 patients and 100 healthy controls. Sonicated Ag, phenol water extract, trichloroacetic acid extract, heat killed and whole cell antigen were used. NCTC *B. fragilis* 2553 was employed in parallel throughout the study. Ag detection was tested by Coagglutination, SDS- PAGE & Haemagglutination. All 273 strains of *B. fragilis* could be correctly identified by Coagglutination. By SDS-PAGE, it was not possible to pick out diagnostic bands for *B. fragilis* group. The seropositivity was 43.91% in Double diffusion, 53.79% in Counterimmunoelectrophorsis, 65.52% in Indirect Haemagglutination, 62.99% in Immnunofluorescent Antibody test. An in-house ELISA test was developed during this study with a positivity 61.15%. A Rapid Indirect Haem Agglutination Slide Test (RIHAST) was also developed, 63.05% was Positive.

Conclusion: Many virulence factors were demonstrated in *B. fragilis* group. Efficacy of the serological tests for detection of Ag & Ab were compared and evaluated. The result of RIHAST was in accordance with the conventional IHA.

DIFFERENTIAL ROLES FOR *BACTEROIDES FRAGILIS* IRON STORAGE PROTEINS DPS AND BFDPSL *IN VITRO* AND *IN VIVO*

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Bacteroides fragilis is a Gram negative obligate anaerobe and member of the normal intestinal flora of humans. When limited to the intestinal tract, *B. fragilis* performs many beneficial functions; however, in the event of damage to the intestinal lining *B. fragilis* can translocate into the oxygenated peritoneal cavity. The resulting immune response to this translocation causes peritonitis and formation of intra-abdominal abscesses. It has been demonstrated that the *B. fragilis* oxidative stress response (OSR) is required for survival in the oxygenated tissue of the peritoneal cavity during abscess formation. One aspect of the *B. fragilis* OSR is the management of intracellular concentrations of ferrous iron (Fe^{2+}). To prevent hydroxyl radical production, proteins from the ferritin family bind available ferrous iron and convert it to the non-reactive ferric iron (Fe^{3+}). *B. fragilis* contains three ferritin family proteins FtnA, BfDPSL, and Dps which belong to the ferritin, bactoerioferritin/DPSL, and Dps ferritin subfamilies respectively. Previous studies have demonstrated a high level of similarity between the Dps and DpsL proteins. Therefore we rationalized that BfDpsL and Dps may be serving similar if not compensatory roles within the cell. To evaluate this we constructed a double Δ*dps* Δ*bfr*(BfDpsL) mutant. We found that this double mutant was considerably more sensitive to oxygen exposure and tert-butyl hydroperoxide during aeration when compared to either of the Δ*dps* or Δ*bfr* mutant alone. Furthermore we were able to complement *bfr* on a multi-copy plasmid and restore resistance to tert-butyl hydroperoxide comparable to Wild type (WT) levels. To further evaluate the role of these proteins we performed competition assays using the Rat Ping-Pong ball abscess model to evaluate the ability of the double mutant to compete with WT. It was found that the double mutant was attenuated in these experiments. Further work is needed to elucidate the roles of BfDpsL *in vivo* and to define the specific roles of Dps and BfDpsL within the abscess. Additional work is also need to define the unique regulation of *bfr* and *dps*.

METABOLIC PROFILING OF THE DISTAL HUMAN COLON USING A CHEMOSTAT MODEL

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The human gastrointestinal (GI) tract is home to a complex ecosystem of diverse microbes, the 'gut microbiota.' Imbalance of this ecosystem can produce abnormal levels of gut-microbial metabolites, potentially contributing to disease. There is much interest in analyzing these metabolites; however, it is difficult to evaluate the metabolic profile of raw fecal specimens. To address this problem, we utilize an *ex vivo* continuous culture model to simulate the human gut microbiota for the purpose of analyzing the metabolic profiles produced by gut microbes.
Our model 'Robogut' chemostat supports growth of microbial ecosystems derived from feces by mimicking the environment of the distal human colon. Elimination of dietary variability allows for comparison between multiple fecal donors. Also, control over substrate input enables the introduction of purposeful perturbations, such as antibiotic treatment. In order to acquire metabolite profiles of the Robogut-supported ecosystems, regularly-obtained samples were subjected to 1D-^{1}H NMR spectroscopy. To validate our model and the application of ^{1}H-NMR to metabolic profiling, we first optimized sample preparation before comparing the profiles of: a) different individuals; and b) the same individual following application of clindamycin. Approximately 40 quantifiable high-confidence metabolites were identified. Principal component analysis of generated metabolite profiles facilitated the comparison of complex datasets by degree of similarity. Our results demonstrate that donor-specific profiles are attainable and reproducible in the Robogut model; that ^{1}H-NMR represents a suitable analysis platform; and microbe-derived metabolites change dramatically in response to antibiotic perturbation. This platform serves as a useful tool for investigating the metabolic profiles in states of gut imbalance, such as the intriguing subset of children with autism that display GI disturbance. We conclude that metabolic profiling carried out in this way is a highly controllable tool that will prove useful for further exploration of gut microbial ecology in human health and disease.

PROTEOMIC ANALYSIS OF OUTER MEMBRANE VESICLES IN *BACTEROIDES FRAGILIS*

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B. fragilis is a member of the commensal flora of the human intestine, but is also frequently found in severe intra-abdominal infections. Outer membrane vesicles (OMVs) of Gram-negative bacteria form an important aspect of bacterial physiology and have a relevant role in bacterial-host interactions. These OMV usually contain OM and periplasmic constituents, including proteins, lipoproteins, phospholipids and LPS. In addition some species can incorporate to their vesicle surface membrane proteins, toxins and signaling molecules. *B. fragilis* release OMV and contain several enzymes in their cytoplasm and the polysaccharide, named PSA, in their surface that mediate immune regulation and disease. The main goal of this study was to describe the OMV proteome in *B. fragilis*. The OMV of the *B. fragilis* strain 630R were isolated after inoculating an overnight culture grown under anaerobic conditions at 37^{O}C into 100 mL of defined media previously described as OMV inducer in the species. The vesicles were separated by ultracentrifugation and the sediment suspended in PBS. Proteins were analyzed using SDS-PAGE gels after coomassie colloidal staining. All bands were excised from the gel and processed for further mass spectrometry analysis (nUPLC-MS/MS). MS identification revealed the presence of several surface proteins, but the most prominent were TonB dependent-family outer membrane protein, enolase-like family, cysteine synthase A and ferritin. Recently, a putative plasminogen-binding protein, Bfp60, and a TonB-dependent protein, both located in the outer membrane in *B. fragilis*, were characterized by our group. The Bfp60 is an alpha-enolase and can recognize laminin-1 and convert plasminogen (Plg) into plasmin. TonB can bind to fibronectin and its inactivation influences *B. fragilis* adhesion. The presence of those proteins in the OMV might contribute to *B. fragilis* virulence and also help the species in the nutrient acquisition.

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EFFECTS OF SUGAR SUBSTITUTES ON THE GUT MICROBIOTA OF MICE

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The purpose of this study is to determine if the sugar substitutes, Sucralose and Aspartame, decrease the overall diversity of the gut microbiota and lead to an obesity associated gut microbiome by increasing the *Firmicutes:Bacteroidetes* ratio. A total of 30 mice were housed in the Central Washington University (CWU) vivarium under standard conditions. All mice were fed *ad libitum* with standard mouse chow and given either a) no sugar substitute (control group), b) one of the two sugar substitutes. Mice supplemented with sucralose consumed at least 5 mg per kg body weight per day over a period of 10 weeks. Mice fed aspartame consumed at least 50 mg per kg body weight per day. Stool from each group was collected weekly and either processed immediately or frozen at -70°C for later use. Two methgods of analysis were used to assess changes in the mouse intestinal microbiota. Denaturing gradient gel electrophoresis (DGGE) was used to determine diversity of the gut microbiome. PCR was used to first amplify the conserved 16S region of all bacteria, then nested DGGE primers were used to amplify the phyla *Bacteroidetes*, and *Firmicutes*. Flourescent *in situ* hybridization (FISH) utilizing phyla-specific probes was also used to quantify changes in the *Firmicutes:Bacteroidetes* ratio. This was done using fluorescently labeled probes for *Firmicutes* (CY5) and *Bacteroidetes* (CY3) as well as a universal bacterial probe (FITC). Preliminary results demonstrated the mice fed sugar substitutes displayed a decrease in metabolic rate. Data is currently being collected for this study, and results will be presented. It is expected that the FISH and DGGE data will show an increase in the *Firmicutes:Bacteroidetes* ratio.

NOVEL INSIGHTS INTO THE ROLE OF TWO EXTRA-CYTOPLASMIC FUNCTION (ECF) SIGMA FACTOR FAMILIES IN MEDIATING OXIDATIVE STRESS RESPONSES BY *BACTEROIDES FRAGILIS*

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The gram-negative anaerobe, *Bacteroides,* is an important component of the normal microbiota but it also is the most frequently isolated anaerobe from clinical samples. When *Bacteroides* escape the gut into more aerated extraintestinal sites abscess formation and bacteremia may occur. A robust oxidative stress response is necessary for maximal virulence. During oxidative stress there is a dynamic change in gene expression but there is a paucity of information on factors that control this response. A set of about 15 extracytoplasmic function (ECF) sigma factors are induced by oxidative stress. Two of these, EcfO and EcfOF, were used as models of ECF sigma factor activity during oxidative stress. EcfO and its anti-sigma factor, Reo, were important for resistance to oxidative stress. EcfO controls a regulon of novel lipoproteins whose distribution is restricted to members of the *Bacteroidetes* phylum. Three of these are members of a NigD family of proteins, first described in *Prevotella nigrescens*. Analysis of mutant strains devoid of these proteins suggest that they work together to resist free radical stress produced by menadione. Affinity tagged fusions of the proteins were used to track their sub-cellular localization to the membrane and further experiments showed that these proteins are glycosylated. A yeast two hybrid assay with a genomic DNA fusion library was employed to identify a protein interaction network of these EcfO response proteins. The role of these genes for oxidative stress resistance is under investigation. EcfOF is located upstream of a gene encoding a putative pyruvate carboxylase. This protein potentially functions as an anti-sigma factor as suggested by the interaction of its N-terminal domain with EcfOF. A microarray gene expression approach was used to identify the regulon of EcfOF and preliminary results have shown that one of the targets of is the GAD operon, encoding a glutaminase (*glsA*) and a glutamate decarboxylase (*gadB*). GadB has been implicated in resistance to low pH stress and preliminary data show that deletion of either *sigOF* or *gadB* from *B. fragilis* results in sensitivity to low pH. This research will help elucidate the roles played by these sigma factors which enable *B. fragilis* to establish opportunistic infections.

EZAKII PERUVIENSIS GEN. NOV. SP. NOV. ISOLATED FROM THE GASTROINTESTINAL TRACT OF AN INDIGENOUS PERUVIAN COMMUNITY

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While there are numerous publications analyzing the gut microbiome of individuals from western populations, diverse human communities are underrepresented in human microbiome studies. In order to truly analyze if there is in fact a core microbiome, individuals with a variety of diets and geographic regions also need to be included. The primary purpose of this study is to test the hypothesis that traditional communities from remote regions harbor novel microorganisms influenced by diet, health, and environmental conditions. A focus of our group was to use RNA-based road maps to target previously uncultivated groups to investigate phylogenetic, physiological, biochemical, and chemotaxonomic properties.

Freshly voided fecal samples were collected from members of the Afro-Peruvian community of Cruz Verde in Tambo de Mora, region Ica, in Peru. Multiple enrichments were prepared and isolates were subcultured to purity on blood agar and then screened using 16S rRNA analysis gene sequence analysis.

A number of isolates yielded relatively low sequence values and phylogenetic tree topologies demonstrated that these belonged to the anaerobic Gram positive cocci. The nearest relatives included members of *Peptoniphilus, Finegoldi, Gallicola* and *Pavimonas*; the isolate representing a novel genus was named *Ezakii* after Takayuki Ezaki who has contributed immensely to the taxonomy of this group of organisms. Our investigations demonstrate that remote indigenous communities harbor novel microbial taxa and further studies employing culture-based approaches of human gut microbiomes of diverse communities are encouraged to augment molecular investigations.

ARSENATE METABOLISM GENES IN ALKALIPHILIC BACTERIA OF SOAP LAKE

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Soap Lake is a permanently stratified (meromictic) lake located in Grant County, WA (USA). The lower layer of this haloalkaline highly sulfidic lake (termed the monimolimnion) exhibits a high concentration of arsenic, ranging from 2.4-2.5 ppm. The purpose of this research is to address three questions about arsenic metabolism by bacteria found in the sediments associated with the monimolimnion layer of Soap Lake. These are as follows: 1) how much arsenate can be tolerated by these populations 2) are arsenic detoxification and respiration genes present in these populations, and 3) will *arr* primers that have worked in past research work for a faster DGGE driven method to quickly assess the diversity of arsenate-respiring bacteria? To accomplish this, sediment samples were collected from the lake, and cultured in defined media under anaerobic conditions with various concentrations of arsenate (5 – 160 mM). Growth was monitored by spectrometry. Positive cultures were analyzed by denaturing gradient gel electrophoresis of 16S ribosomal RNA and arsenate reductase (*arr* and *ars*) gene fragments, followed by sequencing of bands extracted from the gel. The dominant species identified in the arsenate enrichments had the greatest sequence similarity to species in the genera *Alkaliphilus, Thioalkalovibrio, Psuedomonas, Halomonas,* and *Desulfovibrio*. The three *Alkaliphilus* species found tolerated the greatest range of arsenate concentrations, ranging from 10 mM - 160 mM. The dominant arsenate-respiring populations demonstrated greatest sequence similarity to species within the genera *Alkaliphilus* and *Thioalkalivibrio*. While this activity has been reported in the former genus, the ability to respire arsenate has not been previously reported in the *Thioalkalivibrio* genus. In conclusion, populations of alkaliphiles in Soap Lake can tolerate at least 160 mM arsenate, there are a variety of arr sequences found in arsenate-tolerant organisms, and primers utilized in the study of arsenate-respirers were suitable for determining diversity of arsenate-respiring populations via DGGE analysis.

ISOLATION AND CHARACTERIZATION OF NOVEL ANAEROBES FROM THE GUT MICROBIOME OF PACIFIC OYSTERS

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The purpose of this project is to isolate and characterize the novel species of anaerobes found in the natural microbiome of Pacific oysters (*Crassostrea gigas*). Oysters depend on commensal cellulolytic anaerobic bacteria in their digestive tract to break down the cell wall to release the nutrients in their primary food source, algae. We hypothesized that the gut microbiome of Pacific oysters would be a source of novel anaerobes that have not been described before in previous studies, as other studies focused on isolating and characterizing potential human pathogens associated with shellfish consumption. Fresh, live oysters were dissected to remove the intestine and stomach tissues. Tissues were homogenized, diluted, and plated on rich media in anaerobic conditions. Isolated colonies were characterized by growth requirements, Gram stain reactions, and cellulolytic activity. Genomic DNA was isolated from organisms of interest and the 16S rDNA was amplified by PCR, sequenced, and analyzed using comparative phylogenetic methods. To date we have identified numerous novel facultative anaerobic organisms representing *Bacillus spp*., *Eubacterium spp*., *Providencia spp., Enterococcus spp*., *Morganella spp., Shewanella spp., Vibrio spp., Carnobacterium spp., Enterobacter spp., Serratia spp., Photobacterium spp*., and several novel obligate anaerobes representing *Clostridium spp*., and *Proprionibacterium spp*.

ENRICHMENT FOR CELLULOLYTIC ANAEROBES IN THE GUT MICROBIOME OF PACIFIC OYSTERS

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In a previous study, we isolated and characterized a number of novel anaerobic, cellulolytic microorganisms from the gut microbiome of Pacific oysters (*Crassostrea gigas*). Here, we postulated that we could enrich for cellulolytic microorganisms by feeding oysters a defined, concentrated algal food source. Fresh, live oysters were separated into 4 growth conditions: Ocean (dissected upon arrival), Instant Ocean (incubated in sea water for 24 hours at 12°C), Algae 24 (incubated in sea water spiked with algae for 24 hours at 12°C), and Algae 48 (incubated in sea water spiked with algae for 48 hours at 12°C). Oysters were dissected to remove the intestine and stomach tissues. Tissues were homogenized, diluted, and plated on rich media under anaerobic conditions. Isolated colonies were characterized by growth requirements, Gram stain reactions, and cellulolytic activity. Cellulolytic activity was determined by the ability to grow on minimal media in which soluble cellulose is the only major carbon source available. Genomic DNA was isolated from organisms of interest and the 16S rDNA was amplified by PCR, sequenced, and analyzed using comparative phylogenetic methods. We profiled the changes in the oyster gut microbiome with and without algal enrichment. To date we have discovered 10 novel facultative anaerobic microorganisms representing *Bacillus spp*., *Morganella spp*., *Providencia spp*., and *Shewanella spp*. that are capable of cellulose digestion. We have also identified a novel *Propionibacterium spp*. cellulolytic bacterium.

GENOME-WIDE TRANSCRIPTIONAL ANALYSIS OF *FURA* AND *PERA* MUTANTS IN *BACTEROIDES FRAGILIS*

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Bacteroides fragilis is the anaerobe most frequently isolated from human infections such as intra-abdominal abscesses and bacteremia. *B. fragilis* has an essential requirement for iron but the mechanisms involved in the iron acquisition and the regulation necessary to overcome the host iron withholding mechanisms have not been elucidated. Moreover, in bacteria the control of iron metabolism and the oxidative stress response are linked via a number of transcriptional regulators but the role of iron in the oxidative stress response of anaerobes such as *B. fragilis* has not been given much attention. In this study we have used microarray analysis of whole genome transcription expression in the *B. fragilis* 638R *furA* and *perA* deletion mutant strains to understand the iron-responsive regulatory network and the effect of the peroxide response regulator on metal homeostasis. In anaerobic and low iron conditions, 60 genes were up-regulated and 36 genes were down-regulated more than 4-fold in the *furA* mutant compared to 59 genes up-regulated and 35 genes down-regulated in the parent strain compared to iron-replete control cultures. When the *fur* mutant was grown in iron replete conditions, there were 26 genes up-regulated and 9 genes down-regulated more than 4-fold compared to parent strain suggesting that there are Fur-dependent and Fur-independent iron regulatory mechanisms in *B. fragilis*. Moreover, there were 23 genes that were constitutively up-regulated more than 4-fold in the *furA* strain independent of the iron content of the media and whose expression was not significantly changed in the parent strain under high iron or low iron conditions. Interestingly, the FurA homologue PerA did not significantly affect the expression of genes involved in peroxide stress response or iron utilization. Nonetheless, there were 158 up-regulated and 116 down-regulated genes in the *perA* mutant compared to the parent strain under anaerobic condition controls. Taken together, our data demonstrate that metal homeostasis and oxidative stress response in this aerotolerant anaerobe involves a complex genetically regulated response.

IDENTIFICATION OF *BACTEROIDES FRAGILIS* PROTEINS TARGETED BY THE THIOREDOXIN SUPERFAMILY

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The opportunistic pathogen *Bacteroides fragilis* is the most frequent anaerobe isolated from human infections such as, intra-abdominal abscesses and bacteremia. *B. fragilis* withstands long periods of oxygen exposure by inducing a robust and protective oxidative stress response (OSR) against oxidative damage. The thioredoxin redox system consisting of a thioredoxin reductase (TrxB) and six thioredoxin homologues TrxA, D, E, F, G and P with the canonical CxxC motif is known to play a crucial role in its OSR. All *Bacteroides spp*. lack a glutathione system and therefore the couple of TrxB/Trx is the major thiol-disulfide redox system. To understand the role of abundant Trx orthologs in cellular redox balance, site directed mutagenesis was used to construct Trx mutations with CxxS active sites. This mutation allows the attacking active Cys residue to bind the target proteins irreversibly as the second redox Cys residue is absent. All Trx CxxS mutants were fused to a C-terminus His-tag and purified using Co^{++}-agarose affinity resin. Purified Trx-CxxS-6xHis proteins were covalently bound to a cyanogen bromide activated sepharose resin. Then crude extracts of *B. fragilis trx* deletion mutant strains were passed through the different CNBr affinity resins. After washing, the bound target proteins were eluted with 50 mM DTT, concentrated and separated on 10% SDS-PAGE for protein profile analyzes. Protein bands of interest were excised from the gel for LC-MS/MS spectroscopy identification. Thus far, we have identified the major potential targets for the periplasmic TrxP protein as Alkyl hydroperoxidase subunit C, AhpC (BF638R_1276), Thioredoxin peroxidase, Prx (BF638R_2372) and Thiol peroxidase, Tpx (BF638R_2786), Elongation factor Tu, EF-Tu, (BF638R_4059) and Glyceraldehyde 3-phosphate dehydrogenase, GapA (BF638R_0945) and an uncharacterized protein (BF638R_2939). We expect that the identification of additional Trx target putative redox-regulated protein will contribute to our understanding of the thiol-disulfide homeostasis balance in anaerobes during oxidative stress response.

INTERACTIONS BETWEEN THE OPPORTUNISTIC PATHOGEN *BACTEROIDES FRAGILIS* AND HOST PROTEINS

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Bacteroides fragilis is a member of the normal microbiota that resides in the human gastro-intestinal tract. This bacterium is of clinical significance because it is the most frequently isolated Gram-negative obligate anaerobe from peritoneal abscesses and bloodstream infections. Human fibrinogen is a hexameric-glycoprotein that is important for abscess formation and limiting the spread of infection. *B. fragilis* can bind and degrade fibrinogen, which may aid in its escape from abscesses into the bloodstream, thereby promoting bacteraemia. An outer membrane protein, BF1705, expressed by *B. fragilis* was found to share homology with BspA from *Tannerella forsythia* which is known to bind fibrinogen. The gene encoding BF1705 was deleted from the *B. fragilis* NCTC9343 genome using a markerless gene deletion technology. Proteins derived from the outer membranes of wild type *B. fragilis* are able to bind fibrinogen in far western blots. Similar protein extracts from the ΔBF1705 strain did not bind fibrinogen, which confirms the role of BF1705 in interactions with this host protein. Binding of fibrinogen to the surface of ΔBF1705 cells was still observed by immunofluorescence microscopy, which indicates an additional mechanism for host protein interactions. A possible role for capsular polysaccharides in fibrinogen binding is being investigated. To identify the proteases involved in degradation of fibrinogen, several genes encoding putative metallo- and serine proteases were identified and deleted from the NCTC9343 genome. Functional analysis of single and multiple mutants using zymography will be presented.

CHARACTERIZATION OF THE BMOR TRANSCRIPTIONAL REGULATOR, A MEMBER OF MARR FAMILY, IN *BACTEROIDES FRAGILIS*

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Bacteroides fragilis is the anaerobic microorganism most commonly isolated from endogenous infections. The species is highlighted as a major pathogen in anaerobic infections due to its versatility in the relationship with the host, sometimes as a component of the microbiota, sometimes as a pathogen expressing virulence factors. Aerotolerance seems to contribute decisively in the process of interaction with the host and establishment of the infection. *B. fragilis* can survive when exposed to atmospheric oxygen for up to 72h. Under these circumstances, a strong response to oxygen stress is activated and the expression of 45% of *B. fragilis* genome is affected. The MarR family of transcriptional regulators consists of a set of proteins that bind directly to the DNA, controlling a variety of biological processes in bacteria and archaea, including response to oxidative stress, expression of virulence factors and antibiotic resistance. At least 3 members of the MarR family are present in *B. fragilis* strain 638R and, in a previous study, we established that one of them, BmoR, is associated with the oxidative stress response in the species. The aim of this study is to evaluate the role of BmoR in *B. fragilis* virulence and survival in the host using *in vitro* and *in vivo* assays. The inactivation of the *bmoR* gene lead to a reduction in the survival of the mutant strain during interaction with peritoneal macrophage. The inactivation also affected the abscess formation in an intra-abdominal abscess infection model, with reduction in the number of abscesses on the mutant strain. Oxidative stress response assays followed by molecular analysis will be conducted on different strains from our lab's culture collection to correlate the presence of the *bmoR* gene and susceptibility to oxygen. Our preliminary results show that this study may help understand the mechanisms of virulence of *B. fragilis* and still serve as a target in developing new strategies for intervention and control of infections involving this species, even in the medium to long term, given the increasing resistance to antibiotics used in therapy.

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PI-16

COMPARATIVE STUDY OF THE OXYGEN TOLERANCE OF *BACTEROIDES SPP.* IN DIFFERENT CULTIVATION ENVIRONMENTS AND INTERACTION ASSAYS

Lorete, A.R.M.;[1] Dias, M.F.;[1] Ferreira, L.Q.;[1] Fernandes, K.C.B.;[3] Guardiano-Nascimento, C.;[2] Rodrigues, P.S.;[2] Santos-Filho, J.;[1] Lobo, L.A.;[1] Filippis, I.;[3] Seabra, S.H.;[2] Vieira, J.M.B.D.;*[2] Domingues, R.M.C.P.[1]
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The species *Bacteoides fragilis*, *B. thetaiotaomicrom* and *B. vulgatus* are part of the genus *Bacteroides* that is prevalent in amphibiontic microbiota of the intestinal tract. The species *B. fragilis* stands out as an etiologic agent in several human infectious processes such as bacteremia, intra-abdominal infections and abscesses, but the species *B. vulgatus* and B. *thetaiotaomicron* are more related to antimicrobial resistance. As obligatory strict anaerobes, these bacteria would be unable to survive and multiply in the presence of oxygen but it has been demonstrated that the species *B. fragilis* is resistant to oxidative stress, microbicidal mechanism used by macrophages and may remain viable for about 72 hours in the presence of O_2. The oxygen tolerance is related to enzymes involved in the global oxidative stress response that act in the detoxification and consequent protection, for example, as catalase, which is regulated at the transcriptional level by the OxyR protein. In this study tests were performed to evaluate the interference of catalase and OxyR in *B. fragilis* strains survival. Furthermore, *B. vulgatus* and *B. thetaiotaomicron* strains were also analyzed in aerated environments in human blood and after phagocytosis by mouse peritoneal macrophages (MØ). We also assessed the behavior of MØ microbicidal activity after interaction with strains of these species. For both, were performed immunocytochemistry assays for actin and iNOS (inducible nitric oxide synthase) and analysis by electron microscopy and scanning transmission electron microscopy. Preliminary results show different behaviors between the front exposure to oxygen species as well as interference with MØ microbicide action.

1300 ***POSTER SESSION I:*** **MICROBIOTA: REACHING BEYOND THE GUT**

Posters will be presented in Poster Session I
Sunday, June 29 1300-1400.

DIFFERENCES IN ANAEROBE INTESTINAL MICROBIOTA ASSOCIATED WITH WEIGHT GAIN IN CHILDREN

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The intestinal tract is a complex ecosystem, and the relationship between host and microbiota are centralized on the metabolic capabilities of both to find nutrients unavailable or poorly used. Studies have shown that a specific intestinal microbial community may play an important role in the weight gain. In this study, the presence of some anaerobic intestinal bacteria in children with obesity (30), overweight (24) and normal weight (30) was evaluated. Children from 3 to 12 years old were included in this study. Fresh stool samples from these children were collected. Species of *Bacteroides, Parabacteroides* and *Clostridium* were isolated on agar media. Total DNA from stools was extracted and submitted to quantitative analyses by using specific primers to order *Bacteroidales*, *Clostridium* Cluster I, genera *Lactobacillus* and *Bifidobacterium*, and *Bacteroides fragilis, B. vulgatus, Parabacteroides distasonis, C. perfringens, C. difficile*. In most of the stools of all the children groups, *B. vulgatus* and *C. perfringens* were predominantly recovery. Data based in the quantitative detection, showed that in children with normal weight *Bifidobacterium spp.*, *C. perfringens* and *C. difficile* were observed in high concentration; and in children with weight gain species of the order *Bacteroidales* and species of *Lactobacillus* were predominantly detected. Studies have shown that *Bacteroidetes* are commonly decreased in obese individuals, and *Lactobacillus spp.* increased in individuals with weight excess. Our results showed a high concentration of species of the order *Bacteroidales* in both children with overweight and obesity. Studies are necessary to better understand the intestinal microbiota in these evaluated children groups.

OPTIMISATION OF THERAPEUTIC GENE EXPRESSION IN A CLOSTRIDIAL TUMOUR THERAPY DELIVERY VEHICLE

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[3]DNA2.0 Inc, Menlo Park, CA, USA

Clostridial-directed enzyme pro-drug therapy (CDEPT) aims to eradicate tumour cells by using non-pathogenic, anaerobic bacteria, modified to produce a pro-drug converting enzyme able to selectively generate active drug in anoxic tumours.

We have integrated a synthetic gene encoding a novel nitroreductase enzyme (*nmeNTR*) into the genome of *Clostridium sporogenes*. Testing of the therapeutic strain in an *in vivo* tumour model led to a significant tumour regression, and in some cases, to a cure. The objective of the work presented here was to increase the efficacy of our strain by optimising the expression of the therapeutic gene.

A comprehensive investigation of the parameters affecting gene expression was carried out at the molecular level. Both translation and transcription processes were examined. Two libraries, one of promoters, one of ribosome binding sites, were generated and tested. Preliminary results led to the creation of eight promoter-RBS variants, the best of which were selected for chromosomal integration into *C. sporogenes* genome. Additionally, we collaborated with DNA2.0™, in the development of a new algorithm for predicting clostridial codon optimisation.

We measured the level of protein produced by *C. sporogenes* when carrying an expression vector containing one of 60 synthetic variants of the same gene and composed of different combinations of synonymous codons. These data have allowed the formulation of an algorithm that more accurately predicts optimum codon utilisation in this clostridial species.

The combination of promoter, RBS and codon data allowed the generation of a new strain that produces 3-4 times higher nitroreductase activity than the progenitor strain. The next step is to test this strain for pro-drug conversion and tumour regression in a mouse model.

CETOBACTERIUM SOMERAE FIRSTLY ISOLATED FROM CLINICAL SPECIMENS WITH ACUTE CHOLECYSTITIS

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Cetobacterium somerae is a gram-negative obligatory anaerobic rod-shaped bacterium, firstly described in 2003 from human feces (Finegold, S.M.). Although *Cetobacterium spp*. has been isolated from gut flora of fish, there have been no other reports to cultivate it from clinical specimens. By using routine techniques, we could successfully isolate it from bile specimens obtained from a patient with acute cholecystitis.

Patient: 76-year-old female was admitted to our hospital due to 2-day history of nausea, back pain and abdominal pain. She had a history of gastrectomy from gastric carcinoma and gallbladder stone. After the diagnosis of acute cholecystitis with gallbladder and common bile duct stone was made, percutaneous transhepatic gallbladder drainage (PTGBD) was performed, and the patient improved uneventfully with 7-days antibiotic therapy including cefoperazone/sulbactam and meropenem. From blood cultures, *Aeromonas spp*. and *C. perfringens* were cultured, whereas PTGBD bile yielded *Aeromonas spp*., *K. pneumoniae*, anaerobic gram-negative rod (GNR), and *C. perfringens*. The patient underwent a cholecystectomy with choledochotomy on day 23, and eventually discharged.

Microbiological Study: The anaerobic GNR produced gray colonies on Brucella blood agar plates with hemin and vitamin K1 after 3 days-incubation. Although identification kits showed no conclusive results, the isolate was positive for indole, α-galactosidase, β-galactosidase, glutamic acid decarboxylase, and pyroglutamic acid arylamidase. 16S ribosomal RNA sequences gave a 100% concurrence to *C. somerae* (ATCC 27774). It was not a β-lactamase producer and supposed to be susceptible to ampicillin (MIC 0.5 μg/mL), meropenem (0.25), and clindamycin (<0.25), but resistant to levofloxacin (16) by using broth microdilution tests.

Conclusion: This case revealed that longer incubation period would be preferable for culturing *C. somerae* from clinical specimens. Further investigation is needed to clarify its implications in infectious process.

IDENTIFYING THE BACTERIAL ORIGIN OF PROSTATITIS WILL REDUCE THE INCIDENCE OF PROSTATIC BIOPSIES AND PROSTATECTOMIES

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Background: Prostate cancer is the most common cancer in men in United States and it is their third leading cause of death. A high prostate specific antigen (PSA) has been an indicator for prostate biopsy or surgery. This study seeks to detect all type of possible bacteria that can increase PSA value.

Methods: We studied 152 men; 60 healthy and 92 were outpatients particularly symptomatic with a mean age of 63.1 years, and a PSA of 7.82 ng/ml. All had prostatic fluid, urine or semen cultured while searching for: anaerobic, microaerophilic, aerobic, and Chlamydia trachomatis (CT) was done by Immunochromatography. Samples were cultured immediately after collection and incubated at 37°C in different media and atmospheric environments as mentioned above for 5 to 15 days and antibiotic susceptibility test (AST) by the disk diffusion method was done.

Results: Bacterial etiology of the prostatitis was detected in 95.65% of the 92 cases and CT in 40.6%, none had a CT as a unique germ. As single germ, *Staphylococcus aureus* was the pathogen in 58.69% and was present in 6.6% of healthy cases. The sensitivity for bacterial etiology was 95.65%, specificity 71.66%, PVP (predictive value positive) 83.8%, PVN (predictive value negative) 8.5%. The strict anaerobic environment allowed the bacterial isolation in 49.79% of the cultures; they were positive in 16.3%, of which 3.26% were *Bacteroides sp*., 2.17% were *Peptostreptococcus sp*. and 2.17% were *Veillonella sp*. Mixed cultures were 18.47% and negative 4.34%. All treatments were made according to the AST result, none empirically. The prostatic PSA value was reduced after antimicrobial treatment in 53.3% of all prostatitis cases. In one case, having an initial PSA 18.64 ng/ml after cleared of *S aureus* (CT negative) the PSA dropped to 7.2 ng/ml.

Conclusion: There should be a universal protocol when confronted with an elevated PSA. This study shows: 1. Bacterial prostatitis increases the PSA value, 2. The anaerobic cultures are necessary to make a diagnosis of prostatitis, 3. Identifying the bacterial origin and sensitivity, will reduce bacterial resistance, prostatic biopsies or prostatectomies.

PI-21

A CASE FOR *CLOSTRIDIUM PERFRINGENS* EPSILON TOXIN AS A CAUSATIVE AGENT FOR NASCENT LESION FORMATION IN MULTIPLE SCLEROSIS

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Newly forming (nascent) Multiple Sclerosis (MS) lesions are remarkably specific and their distinct histopathologic characteristics provide important clues about the offending agent. These characteristics are: 1) focal defects in the blood-brain barrier; 2) oligodendrocyte cell death; 3) early preservation of myelin; 4) preservation of neurons; 5) absence of an astrocytic response; and 6) early activation of microglia. Among the long list of proposed environmental triggers for MS, there are no proposed environmental agents that mechanistically explain these findings and no animal models that recapitulate the essential elements of the nascent lesion. We propose that *Clostridium perfringens* ϵ-toxin is the causative agent responsible for nascent lesion formation in MS. Following IV injection of fluorescently tagged ϵ-toxin, specific intraluminal binding of ϵ-toxin is observed in microvasculature of the brain, retina and intestine as previously reported. In primary CNS cell cultures and in organotypic cerebellar slice cultures, *Clostridium perfringens* ϵ-toxin binds to oligodendrocytes and myelin but not to neurons, astrocytes, or microglia. *Clostridium perfringens* ϵ-toxin induces demyelinationin cerebellar slice cultures in a dose and time dependent fashion. Oligodendrocyte death precedes demyelination in ϵ-toxin treated cerebellar slices. Neutralizing antibodies directed against *Clostridium perfringens* ϵ-toxin prevent oligodendrocyte injury and demyelination. In conjunction with the recent finding of prior exposure to ϵ-toxin in people with MS, these findings further implicate *Clostridium perfringens* ϵ-toxin as an environmental agent responsible for nascent lesion formation in MS.

1300 POSTER SESSION I: BENEFICIAL MICROBIOME MEMBERS

Posters will be presented in Poster Session I
Sunday, June 29 1300-1400.

ASSESSMENT OF THE KNOWLEDGE AND PERCEPTION OF PROBIOTICS AMONG MEDICAL SCIENCE STUDENTS AND PRACTITIONERS IN LAGOS STATE

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Probiotics are live organisms that confer health beneficial effects to the host when consumed in controlled and adequate amount. Their role as therapeutic agents for the prevention and management of gastrointestinal infections, allergic diseases and their anticancer potentials is globally recognized. There is a need to assess the level of knowledge among health professionals and students on the use of probiotics. We conducted a questionnaire based survey using a predesigned pilot tested tool, to assess the knowledge and perception of probiotic among medical science students and health practitioners in Lagos state. The questionnaires were randomly administered to 270 medical science students and professionals from various health institutes in Lagos state. The knowledge level was scored 0-3 as poor, 4-6 as fair and 7-9 as good and analyzed using Epi info version 3.5.3. Of the 270 questionnaires distributed, 265 (98.1%) were returned by 164 medical science students and 101 practitioners. The knowledge score of medical science students and practitioners was low (Mean± SD of 3.62 ± 2.7). 94 (57.3%) students disclosed that they have never heard of probiotics before and 139 (84.8%) indicated interest in knowing more. 70 (69.3%) practitioners were familiar with the term probiotics but 42 (41.6%) had poor knowledge. 73% were not aware of any proven probiotic product in Nigeria and none has prescribed probiotic products for any medical condition. All indicated interest in knowing more about probiotics. The comparison of knowledge result across the various groups was statistically significant ($P<0.05$). There is limited knowledge and poor perception on benefits of probiotic use among medical science students and professionals in Lagos state. The result of this study indicates a need for education on the availability, sources and benefits of probiotics.

EFFECTS OF POTENTIAL PROBIOTIC STRAINS ON MECHANISMS OF HOST DEFENSE

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Probiotics are believed to enhance the host defense through a variety of mechanisms including stimulation of both the innate and the adaptive immune systems. Reactive oxygen species (ROS) as NO and H_2O_2 could play an important role in the first line of host defense as inducers of oxidative stress.

In this study two potential probiotic strains, *L. salivarius* (C3) and *L. rhamnosus* (C44), isolated from infant's gut microbiota, were examined for their ability to stimulate large intestine epithelial cells (Caco2) and peripheral blood mononuclear cells (PBMCs) by releasing ROS. Both live and heat inactivated (C3HI, C44HI) lactobacilli were investigated. *Lactobacillus rhamnosus* GG was used as probiotic reference strain. The cytotoxicity of potential probiotics to PBMCs was measured with trypan blue staining assay. The enhancement of mitochondrial activity of Caco2 cells was estimated with MTT assay. The cells' release of NO after stimulation by lactobacilli was determined according to the Griess reaction, while released H_2O_2 was estimated photometrically after addition of peroxidase and MTT solution.

Only C44HI increased the mitochondrial activity in Caco2 cells compared to the control (Caco2 cells not exposed to the lactobacilli). Heat inactivated and the control strain, but not live lactobacilli (C3, C44), provoked significant NO production. The H_2O_2 production from Caco2 cells was enhanced significantly after their incubation with C3, C44 and LGG.

The presence of potential probiotics did not exert any cytotoxicity effect to PBMCs. Only the LGG strain induced a higher NO release compared to control. The H_2O_2 production from PBMCs was enhanced significantly after their incubation with C3HI and C44HI lactobacilli.

These results indicate that the tested strains are not harmful when exposed to human cells and that they induce moderate H_2O_2 production in intestinal epithelial cells (Caco2), a property that might help the host's defense against enteric pathogens.

PI-24

THE IMMUNE PROFILE OF A THERMOSTABLE VAGINAL PROBIOTIC FILM

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A considerable challenge to the global application of vaginal probiotics is the demand for long-term stability and shelf life at a wide temperature range. Our goal is to design a probiotic, which would deliver viable bacteria with favorable immune properties. *Lactobacillus jensenii*, *Lactobacillus rhamnosus* and *Lactobacillus crispatus* predominant in the healthy human mucosal environment were preserved by vaporization drying and micronization (PBV), and subsequently encapsulated into a quick-dissolve film formulation. The stability of the PBV bacteria prior to film incorporation was determined by consistent colony forming unit (CFU) recovery from bacteria stored at room temperature for a year and survival of at least one hour at 70°C and at least 3 months at 37°C. Stability of the PBV bacteria encapsulated in quick-dissolve film and stored at ambient temperature for up to one year was assessed by CFUs and epithelial adherence. Makers of immune function, e. g. NF-κB nuclear translocation and levels of secretory leukocyte protease inhibitor (SLPI), interleukin (IL)-1RA, IL-6, IL-8, IL-1β, and tumor necrosis factor (TNF)-α, were measured in cell culture supernatants and lysates. The proinflammatory effects of live lactobacilli were compared to those of heat-killed bacteria to assess the importance of bacterial viability preservation in probiotic formulations. Epithelial adherence rates of the PBV-preserved bacteria derived from the film were not significantly different from those of cryopreserved strains. CFUs were reproducibly recovered in multiple experiments with both epithelial monolayers and 3D stratified tissue constructs independent of bacterial strain or performing technician. The films with PBV bacteria and a bacteria-free placebo film induced similar low levels of innate immune mediators as compared to toll-like receptor ligands used as pro-inflammatory controls. NF-κB activation in response to proinflammatory stimuli such as double-stranded RNA was enhanced in the presence of heat-killed but not viable lactobacilli. These preclinical data underscore the importance of optimal lactobacillus preservation and demonstrate that the PBV technology coupled with the quick-dissolve film technology can generate probiotic candidates compatible with the vaginal epithelial barrier function.

1300 ***POSTER SESSION I:*** **ANAEROBES IN THE ORAL CAVITY**

Posters will be presented in Poster Session I
Sunday, June 29 1300-1400.

CHANGES IN STREPTOCOCCI AND LACTOBACILLI BEFORE AND AFTER TREATMENT OF EARLY CHILDHOOD CARIES

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Severe early childhood caries (S ECC) are a prevalent public health problem among preschool children throughout the world. They are closely related to high numbers of mutans streptococci and lactobacilli, but little is known about the impact of the the treatment of this disease on the shift in microbiota species found in dental plaque and/or saliva.

The aim of this study was to investigate the effect of treatment under general anesthesia on the microbial changes and their sustainability.

Saliva and plaque samples were collected from 20 healthy infants aged from 2 to 6 years, with ECC, consulting our clinical services (CHU of Bordeaux, France). The samplings were done before and in regular intervals up to 6 months after treatment. Microorganisms were detected by cultivation on selective agar (Mitis Salivarius Agar and MRS Agar) and scored. They were identified phenotypically (Api 20Strep and Api 50CH) and genotypically (PCR method with specific primers). Parents were repeatedly interviewed regarding the children's diet and oral hygiene, accompanied by corresponding advice.

Our results showed that Lactobacilli were mainly recovered in plaque samples among only half of the children. When present in the first sampling, they disapeared after treatment.

Among the identified streptococci, *Streptococcus mutans*, *Streptococcus mitis* and *Streptococcus salivarius* were predominant, showing more diversity than in other studies. No significant changes were found before and after treatment. As nutritional and oral hygiene habits changed only slightly despite advising, this result could be associated with this lack of changes.

So we can conclude that ECC is a complex infection, with influences from selected bacteria, from diet or from oral hygiene habits.

ISOLATION AND IDENTIFICATION OF BACTERIA FROM THE SUB-GINGIVAL PLAQUE OF HORSES

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Oral diseases in horses are a common problem. While there are numerous reports on the dental anatomy and clinical aspects of oral disease in horses, there are very few reports in the literature on the oral microbiology associated with health or disease. Therefore the aim of this study was to isolate and identify cultivable bacteria from sub-gingival plaque of horses using culture and identification using 16S rRNA sequencing.

A dental examination was performed and the animals classified into either healthy or diseased groups. Plaque samples were taken from the gingival sulcus of six horses, four with clinically healthy gingiva and two with periodontal disease. Plaque samples were plated onto Wilkins-Charlgen Agar (WCA) supplemented with 5% defibrinated blood, incubated at 37°C for up to 7 days in an anaerobic chamber (atmosphere of 90% N_2/ 5% CO_2/5% H_2) Up to 30 bacterial isolates from each plaque sample were identified by conventional phenotypic tests and 16S rRNA gene sequencing. Bacterial DNA was extracted using Prepman Ultra® amplified by PCR using the universal primers (27f and 1492r) and the sequence were compared with 16S rRNA gene sequences from Genbank sequence database; The National Centre for Biotechnology Information Website (http://www.ncbi.nlm.nih.gow/BLAST).

The results show that bacteria from 16 genera were isolated from the plaque of healthy horses and 11 genera of bacteria from the diseased. The most common isolates being Veillonella (12%), Gram positive anaerobic cocci (12%), Streptococcus (9%), Porphyromonas (6% include *P. asaccharolytica* 3%, *P. uenonis* 1%), Fusobacterium (7% include *F. equinum* 1%, *F. nucleatatum* 1%), Actinobacillus (6%) and Prevotella (5% include *P. intermedia* 2%, *P. dentasini* 2%) and a number of "uncultured" species/ clones. The preliminary results from the present study showed that equine oral microbiology in health and periodontitis is highly diverse with a higher diversity associated with health. A number of isolates were identified as "uncultured" clones highlighting the high potential for isolation of novel species.

CLINICAL SIGNIFICANCE OF ORAL CAMPYLOBACTERALES

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Many members of the order *Campylobacterales* pose a potential pathogenic risk. The purpose of this study is to search for so far unidentified members in the human oral cavity and to elucidate their role in health and disease.

Methods: Subgingival plaque acquired from periodontitis patients and healthy individuals was subjected to a newly developed nested PCR approach. First, the 16S-rRNA gene of all Epsilonproteobacteria was pre-amplified. In a second PCR, either *Helicobacteraceae* (*Helicobacter spp.* and *Wolinella spp.*) or *Campylobacteraceae* (*Campylobacter spp.* and *Arcobacter spp.*) were specifically and separately amplified. Coamplified 16S-amplicons were separated by SSCP gel electrophoresis and identified by sequencing of excised bands. Finally, confirmation PCRs were performed addressing genus-specific structural genes.

Results: Members of *Helicobacteraceae*, including *H. pylori* (with conflicting data about the oral cavity as a niche for stomach (re-) infection), were only rarely found in our collection of oral samples. Instead, in 11 out of 104 oral samples we could detect a member of the *Wolinella* genus with high sequence identity (98%) to "*Candidatus Wolinella africanus*" which has never been described in the oral cavity before and might be responsible for cases of *H.pylori* misidentification. In contrast, the *Campylobacteraceae* community showed a high prevalence and was composed of typical species like *Campylobacter concisus, C. gracilis, C. rectus,* or *C. showae*, but also of a few new phylotypes. Interestingly, a shift in species composition here reflects changes in the whole microbial community from health to disease.

Conclusions: Although oral bacterial community studies with a universal approach have been done extensively, the focus on members of the *Campylobacterales* preferred in this study reveals the presence of so far overlooked species, relativizes the prevalence of *H. pylori* in the oral cavity and approves *Campylobacter* species as marker bacteria for ecological changes. This could help to assess the pathogenic potential of this order and elucidate the role in local and systemic health and disease.

CARIOGENIC ACTIVITY OF *SCARDOVIA WIGGSIAE* AND *STREPTOCOCCUS MUTANS* IN EARLY CHILDHOOD CARIES

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Background: Anaerobic culture of early childhood caries (ECC) yielded a new caries-associated species, *Scardovia wiggsiae* (Sw), in addition to *Streptococcus mutans* (Sm), non-mutans streptococci, actinomyces and bifidobacteria.

Objective: To evaluate (i) the cariogenicity of Sw in an animal model, and (ii) to explore whether Sw genes were actively expressed samples from ECC compared with caries-free children.

Methods: (i) *S. wiggsiae, S. mutans*, and a combination of both species were inoculated into rats which were fed a high sucrose diet. Colonization was tested by qPCR of rat saliva samples. Caries was scored at 90 days and compared between inoculation groups. (ii) RNA was purified from plaque collected from 2-6 year-old children. Samples were sequenced using the Illumina MiSeq (ECC n=5, caries-free n=5) and gene expression analysis was performed by aligning sequences to genome sequences in the Human Oral Microbiome Database and counting total hits to genes.

Results: (i) Sw colonized rats at low levels, with increased colonization when combined with Sm. The combination of Sw with Sm induced caries at a similar level to Sm, but only minimal caries was detected in Sw only animals. (ii) Multidimensional scaling of gene counts grouped children into caries or caries-free groups. Over and under expressed genes were detected but none differed significantly between ECC or caries-free plaques (FDR < 0.1). Sm (p=0.008) had over expressed genes in ECC compared with caries-free, as did Sw and several other taxa, but not significantly. Over expressed genes included L-lactate dehydrogenase, glucosyltransferase GtfG, surface antigen-related protein, glycogen phosphorylase and lantibiotic streptococcin A-FF22 precursor, all in *S. mutans*.

Conclusion: *Scardovia wiggsiae* showed minimal cariogenicity in an animal model devised for *S. mutans*. *S. wiggsiae* showed an increase in gene expression activity in plaque samples, but at a lower level than *S. mutans*. *S. wiggsiae* does not appear as cariogenic as *S. mutans*, but may play a role in the complex microbial community associated with childhood caries.

PI-29

SURVEY OF GINGIPAINS AMONG *PORPHYROMONAS GINGIVALIS* CLINICAL STRAINS

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Objectives: Adult periodontitis is a chronic destructive inflammatory disease that affects gingival tissue, cementum, periodontal ligament and the alveolar bone. One major pathogen associated with severe adult form of periodontal disease is *Porphyromonas gingivalis*. This organism produces potent cysteine proteases, one major virulence factor known as gingipains, which specifically cleave proteins after arginine or lysine residues. Promises of developing vaccines against periodontal diseases targeting at gingipains were reported in various animal models including murine lesion model, murine bone loss model, rat ligature model or macaque periodontitis model.

Methods: In this study we set out to survey the lysine and arginine gingipains by PCR in over a dozen of clinical *P. gingivalis* isolates. The API ZYM test was performed to examine enzymatic activities of these clinical strains. Protease and haemagglutination assays were carried out to functionally characterize the gingipains.

Results: Our data showed that the catalytic domain as well as the adhesion domains from both lysine and arginine gingipains are highly conserved among strains with 94% protein sequence identity and 97% protein sequence conservation. The API ZYM test confirmed similar patterns of enzymatic activities shared between clinical isolates with *P. gingivalis* W50. Protease assays and haemagglutination assays evidenced comparable levels of both activities to that of W50. The expression of gingipains in these clinical strains was also examined by Western Blotting using gingipain-specific rabbit antiserum.

Conclusion: Our data supports targeting gingipains by vaccination given these molecules are conserved amongst clinical strains.

1300 STUDENT PRESENTATION POSTERS

Judging for Student Presentations is
Sunday, June 29 1200-1300.

Posters will be presented in Poster Session I
Sunday, June 29 1300-1400.

CHARACTERIZATION OF THE CULTIVABLE HUMAN GUT MICROBIOTA BY CULTURE-ENRICHED MOLECULAR PROFILING

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It is widely accepted that up to 80% of the human gut microbiota is unculturable, largely due to the abundance of anaerobic bacteria. As a result, much of the work characterizing the intestinal microbiota has utilized culture-independent molecular methods. However, this does not distinguish between DNA from viable or dead cells, often lacks accurate taxonomic resolution to the species level, and provides little functional information. Culturing bacteria allows for the determination of the viable population, for phenotypic characterization (including rare organisms that may be missed by molecular methods) and provides a valuable resource for further experiments. The purpose of our work was to develop and apply a method of culture-enriched molecular profiling to successfully culture the majority of the human gut microbiota. Fecal samples from five individuals were cultured using 30 different media incubated aerobically and anaerobically. Resultant growth on plates was subsequently analyzed by Illumina sequencing of the 16S rRNA gene and compared to the 16S rRNA gene sequencing of the fecal sample, allowing us to determine the proportion of the gut microbiota captured by culturing. We successfully cultured at least 80% of the species-level phylotypes present at greater than 0.1% abundance in each fecal sample, including most of the anaerobic population. Additionally, we found substantial differences in the cultured communities of fecal samples stored anaerobically compared to aerobically, and between fresh and frozen fecal samples. These findings demonstrate the necessity for proper handling of fecal samples before cultivation, and allowed us to develop a methodical protocol for culture-enriched molecular profiling. This is the first time that a majority of the intestinal microbiota has been shown to be readily cultured. The ability to obtain cultured isolates of intestinal bacteria will be critical for future studies attempting to elucidate the role of specific members of the gut microbiota in both health and disease states.

URINE IS NOT STERILE: THE URINARY MICROBIOTA OF OVERACTIVE BLADDER PATIENTS

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Background: Contrary to dogma that urine is sterile in the absence of a clinical urinary tract infection (UTI); our research team and others have recently shown the existence of a urinary microbiota in individuals with and without lower urinary tract symptoms. With the knowledge that the lower urinary tract possesses its own unique microbiota, we are exploring potential causes for lower urinary tract syndromes, such as overactive bladder syndrome (OAB), a disorder affecting ~15% of adult women. OAB is characterized by symptoms of urinary urgency, often with frequency and urgency incontinence, nocturia and a negative urine culture. ~40-50% of OAB patients do not respond to conventional anti-muscarinic drug treatment. One possible explanation for this lack of treatment response is a dysbiotic urinary microbiota.

Materials: Following Loyola institutional review board (IRB) approval for all phases of this project, women undergoing OAB treatment and a comparison group of women undergoing benign gynecologic surgery (controls) gave research consent for the collection and analysis of their urine. Urine samples from OAB (42) and control (42) were examined by both standard and expanded quantitative urine culture (EQUC) techniques. Matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry was used to classify bacterial isolates.

Results: 60 of the 84 urine specimens (71.4%) grew bacteria. However, 90% of these specimens were deemed "No Growth" by the standard urine culture technique, highlighting its limitations. OAB patients had more diverse urinary microbiota than did control patients: a total of 217 bacterial isolates from 77 different genera were isolated from OAB patients, while 66 bacterial isolates from 33 different genera were isolated from control patients. Organisms isolated solely from OAB patient urines included *Actinobaculum schaalii, Aerococcus urinae, Arthrobacter cumminsii,* and *Oligella urethralis*; each has been reported to cause UTI.

Conclusions: Women with OAB have more diverse urinary microbiota than control patients. This diversity and/or the presence of specific organisms could contribute to OAB symptoms.

A MOLECULAR ANALYSIS OF OXALATE-DEGRADING INTESTINAL BACTERIA IN BLACK AND WHITE SOUTH AFRICANS

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The incidence of kidney stone disease in the South African black population is extremely rare, while that in the white population is comparable to moderate/high risk populations in other countries. Since the majority of kidney stones are composed of calcium oxalate, we aimed to examine whether an enhanced diversity or abundance of oxalate-degrading bacteria in the gastrointestinal tracts of black South Africans plays a role in the low risk of kidney stone formation in this group. Stool samples were obtained from healthy black (n = 20) and healthy white (n = 20) South African male volunteers. An analysis of Oxalobacter formigenes, Lactobacillus and related *spp*. and Bifidobacterium *spp*. present was carried out using DGGE- and qPCR-based approaches. In addition, an aliquot of each stool sample was processed anaerobically in MRS medium to generate a pool of viable bacteria for each sample. The ability of these pools to degrade oxalate was assessed *in vitro* and individual oxalate degrading strains were selectively isolated, one of which was characterised further in terms of its probiotic potential. Samples from the black population showed a greater diversity of Lactobacillus *spp*. and a higher relative abundance of Bifidobacterium *spp*. than those from the white group, while *O. formigenes* was present only at very low levels in either group. Bacterial pools prepared from samples provided by black volunteers degraded oxalate more efficiently *in vitro* than the corresponding pools from white volunteers. A potential probiotic strain of *Lactobacillus gasseri* (strain $B7_2$) isolated from the stool of a black volunteer was able to degrade oxalate and showed good colonisation potential *in vitro*. In conclusion, the South African black population harbours a diverse and abundant pool of potential oxalate-degrading bacteria that may help to protect this group from developing kidney stones.

GUT MICROBIOTA OF LEBANESE PRETERM INFANTS WITH AND WITHOUT NECROZITING ENTEROCOLITIS

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Compared to full term infants, gut bacterial colonization, recognized to play a pivotal role in the development of necroziting enterocolitis (NEC), is severely delayed in preterm infants (PI). This impaired initial colonization is influenced, especially in PI, by several factors including environmental conditions. No relevant data on this subject are available in Lebanon. This study aimed at investigating gut microbiota in Lebanese PI during the first month of life. We included five PI developing NEC matched with five PI non-developing NEC with regards to gestational age (GA) and postnatal age. Fecal samples at d28 of life were analyzed using culture, real-time PCR (qPCR), and Temperature Temporal Gel Electrophoresis (TTGE). The 10 PI were born at a mean GA of 31.5 weeks [27-34]. They showed an impaired bacterial colonization. At about one month all PI were colonized by aerobic bacteria, i.e. staphylococci, enterococci, and enterobacteria. By contrast, regarding anaerobic genera, very few PI were colonized by either *Clostridium* (n=1) or *Bifidobacterium* (n=5), and none were colonized by *Bacteroides*. TTGE profiles revealed few bands per subject, confirming the delayed bacterial colonization. When both NEC PI and non-NEC PI were compared, the difference concerned bifidobacterial colonization with none of the NEC-PI colonized by bifidobacteria by contrast with 3 non-NEC infants colonized (median 4.4 $\log_{10}$ CFU/g feces) using culture method. However, bifidobacteria were detected by qPCR in 2 NEC-PI. Such detection could be due to the presence of bacterial DNA or non-viable bacteria. Likewise, lactobacilli were detected in 3 PI (2 NEC and 1 non-NEC PI) only by qPCR. *Clostridium perfringens*, a species involved in enterocolitis, was detected by both methods only in one NEC infant at a high level (8.6 log/g of feces). We confirm the great impairment of bacterial colonization in Lebanese PI as previously described in other countries. Differences in the colonization profiles between NEC and non-NEC PI should be confirmed in a larger study.

EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE* INFECTION IN PATIENTS TRANSFERRED FROM LONG-TERM CARE FACILITIES TO AN ACUTE-CARE HOSPITAL

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Objective: To understand the epidemiology of *Clostridium difficile* infection (CDI) in patients transferred from long-term care facilities (LTCFs) to an acute-care hospital in Detroit.

Methods: A prospective case-control study was conducted on patients admitted with CDI to a tertiary care hospital in Detroit between October 2012 and October 2013. Data about potential risk factors were collected within 60 days prior to CDI. Patients were then followed for one year by telephone interviews. Measures of outcomes included length of stay, 90-day readmission and CDI recurrence. CDI recurrence was defined as lab confirmed CDI >2 weeks and ≤ 8 weeks after a patient's most recent lab confirmed CDI.

Results: The cohort included 96 CDI patients with a mean age of 56 ± 18 years; 55 (57%) were males, 70 (73%) were African Americans and 16 (17%) were cancer or transplant patients. Twenty two patients admitted from LTCFs were compared to 74 patients admitted from home. Patients admitted from LTCFs were almost 4 times more likely to receive 2 or more antibiotics prior to admission compared to those admitted from home (86% vs 59.5%, p=.02). The median number of total antibiotic days prior to CDI in patients admitted from LTCFs was higher than that of patients admitted from home ({9, Interquartile range {(IQR}) [2.75 – 13] vs 2.5, IQR [0 – 7.25], p=.01)}. LTCF CDI patients were more likely to receive laxatives than CDI patients admitted from home (95.5% vs 63.5%, p=.004). In multivariate analysis, prior use of laxatives was an independent predictor of CDI in patients admitted from LTCFs (OR= 8.72, 95% CI [1.01 – 71.30], p=.04). Although not statistically significant, patients admitted from LTCFs had higher CDI recurrences than those admitted from home (23% vs 12%, p=.22).

Conclusions: This study validates the use of laxatives as a risk factor for CDI patients from LTCF. Given the higher prior antibiotic use and higher length of antibiotic exposure in CDI patients admitted from LTCFs, this study highlights the importance of strictly enforcing antibiotic stewardship programs in LTCFs.

THE ROLE OF NICHE EXCLUSION BY THE GUT MICROBIOTA IN *CLOSTRIDIUM DIFFICILE* COLONIZATION RESISTANCE

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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 via perturbation of the indigenous gut microbiota. Previous studies have demonstrated that colonization of several enteric pathogens is excluded by the resistant microbiota through prevention of access to a preferred nutrient niche. This principle may also extend to *C. difficile*, however it has been difficult to extract specific interactions thus far as no single element of exclusion can been demonstrated. To address this problem, we apply genome-scale metabolic modeling of *C. difficile* in contest with other enteric bacterial species to identify signatures of nutrient competition and provide evidence of strong competitors for the niche of *C. difficile*. To test the validity of *in silico* findings, this defined bacterial consortium, as well as reduced permutations intended to selectively yield nutrient niches, have been examined for *C. difficile* colonization susceptibility in a gnotobiotic mouse model of infection. Thus far, this method has further supported established antagonistic species of *C. difficile* colonization as strong competitors, as well as identified a consortium of other strains that putatively exclude the pathogen from the gut environment based on nutrient competition. The expectation is that yet unappreciated elements of *C. difficile* colonization resistance, particularly those involving nutrient competition, will become more evident with further analysis. Subsequently, more targeted assays of identified metabolite subsets will be employed to conclusively demonstrate competition as a key factor in the exclusion of *C. difficile* from the mammalian gastrointestinal tract. In the future, these methods may be applied to the intelligent selection of probiotic bacterial strains that prevent the colonization of enteric pathogens based on a nutrient niche.

PRE-COLONIZATION BY A LESS VIRULENT STRAIN OF *CLOSTRIDIUM DIFFICILE* PROTECTS FROM RE-INFECTION BY A LETHAL STRAIN

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Background: *Clostridium difficile* infection (CDI) is a major healthcare burden responsible for over $4 billion in excess healthcare costs. Following colonization, *C. difficile* produces two main toxins, TcdA and TcdB, which mediate disease. Clinical studies have demonstrated an association between the development of a humoral immune response towards *C. difficile* toxins and protection from disease. Additionally, studies in a hamster model of CDI show that colonization with a non-toxigenic strain of *C. difficile,* prior to challenge with a virulent strain, prevented disease. In this study, we sought to examine the relative role of bacterial competition versus development of anti-toxin antibodies in meditating protection from CDI.

Methods: Five-six week old female C57BL/6 mice were given 0.5 mg/ml of cefoperazone in their water for ten days, followed by two days sterile water. Four cages of mice were challenged with *C. difficile* 630, while two cages were mock challenged. Six weeks post challenge; mice were injected with 10 mg/kg of clindamycin (to disrupt the microbiota). The next day, mice were challenged with *C. difficile* VPI 10463.

Results: Following challenge with *C. difficile* 630, three cages of mice remained colonized with 10^7 cfu/g feces throughout the experiment. One cage cleared the infection. Clearance was associated with significant alterations in the gut community structure. All mice infected with *C. difficile* 630 developed serum IgG to TcdA while the serum IgG response to TcdB was significantly less robust. Following challenge by *C. difficile* VPI 10463 all mice previously infected with *C. difficile* 630 remained healthy. Only naïve mice became moribund and succumbed to infection.

Conclusions: Previous colonization with a less virulent but toxigenic strain of *C. difficile* provides protection from subsequent infection with a more virulent strain. These data suggest that the development of an adaptive immune response to *C. difficile* toxins and not bacterial competition between strains is important for mediating protection. Additionally, infected mice developed significantly higher levels of IgG to TcdA than TcdB.

UNUSUAL GLUCOSYLATION PATTERN IN TOXIN B FROM A *CLOSTRIDIUM DIFFICILE* NAP1 STRAIN

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NAP1 strains are responsible for *Clostridium difficile* nosocomial outbreaks worldwide. The increased pathogenic potential of strains from this genotype has been attributed to hyperproduction of *C. difficile* toxins and to a more efficient intracellular processing of toxin B. During a cytotoxic screen performed on a collection of NAP1 strains, we identified an isolate whose Cytopathic Effect (CPE) was different from the classic arborizing CPE. Consistent with its NAP1 type, this particular strain hyperproduces toxin A and toxin B, harbors the binary toxin, has a deletion in the PaLoc negative regulator *tcdC,* and is resistant to fluoroquinolones.

Cells intoxicated with purified toxin B from this strain were completely rounded and detached easily from the surface, resembling the effect induced by toxin B from toxin A-negative strains. The substrate specificity of purified toxin B was determined *in vitro* using recombinant small GTPases labeled with radioactive UDP-glucose and in intact cells using GTPase pull down assays. Whereas toxin B from classic NAP1 strains glucosylates Rho, Rac and Cdc42, toxin B from the variant NAP1 strain did not act on Rho and modified Cdc42 less efficiently. On the other hand, the latter toxin glucosylated Rap and R-Ras more efficiently than toxin B from classic NAP1 strains.

A comprehensive genomic characterization has been undertaken to understand the emergence of variant toxins within the NAP1 type. The use of this kind of variant strain in virulence models will help to unravel the role of glucosylation of particular small GTPases in the pathogenesis of *C. difficile*-induced diseases. In addition, these variant toxins would be valuable tools for cell biology studies on GTPase function.

SP-9

A ROLE FOR INTERLEUKIN-23 IN NEUTROPHIL RECRUITMENT DURING *CLOSTRIDIUM DIFFICILE* COLITIS

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Clostridium difficile (*C. difficile*) causes colonic inflammation in antibiotic-pretreated mice, which is characterized by increased expression of interleukin-23 (IL-23), increased expression of CXC chemokines, and robust neutrophil recruitment. We used a murine model of acute severe *C. difficile* colitis to examine the role of IL-23 signaling in neutrophil recruitment. Wild-type and IL-23 deficient ($p19^{-/-}$) mice were treated with cefoperazone (0.5g/L) in their drinking water for five days in order to induce susceptibility to *C. difficile* infection. Following a two-day recovery period, mice were challenged with approximately $5*10^5$ spores of *C. difficile* strain VPI 10463. Infection was allowed to progress for two days, at which point colonic leukocytes and RNA were collected. Recruitment of $CD11c^{Lo}$ $CD11b^{Hi}$ $Ly6G^{Hi}$ neutrophils was significantly reduced in IL-23 deficient animals as compared to wild-type. Consistently, colonic expression of the neutrophil chemokines CXCL1 and CXCL2 was also significantly decreased in the absence of IL-23 signaling. Taken together, these data strongly suggest that IL-23 signaling is required for the full expression of neutrophil chemokines and neutrophil recruitment during *C. difficile* colitis.

1250 POSTER SESSION II: BIOFILMS IN ANAEROBIC INFECTIONS

Posters will be presented in Poster Session II
Monday, June 30 1250-1350.

THE MAKING OF A MISCREANT—METATRANSCRIPTOMIC ANALYSIS OF SMOKE-CONDITIONED BIOFILMS

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Purpose: Purpose of the present investigation was to use an open-ended metatranscriptomic approach to comprehensively explore the gene expression profiles of multispecies biofilms in smoke-free and smoke-conditioned environments.

Methods: Commensal and pathogen-rich biofilms were generated by seeding cover glass slips with primary colonizers, bridge species and tertiary colonizers in a specific temporal sequence to mimic the oral colonization in health and disease. The biofilms were created in smoke-free and smoke-conditioned environments with 1% cigarette-smoke extract. Total RNA was isolated, mRNA extracted from 24-hour biofilms and fractionated. mRNA was used to synthesize cDNA, which was sequenced using Illumina 250bp paired-end sequencing. Filtered sequences were analyzed using the MG-RAST metatranscriptomic pipeline and functional analyses performed at various subsystem levels.

Results: Globally, the most significant effects of smoking were observed on primary and secondary colonizers, but not on tertiary colonizers (pathogen-rich biofilms). In commensal-rich biofilms, smoke-conditioning downregulated genes responsible for adhesion, invasion, intracellular resistance, protein secretion systems, siderophores, quorum sensing, and transposon elements. In commensal-rich biofilms colonized with *Fusobacterium nucleatum* (bridge species), smoke-conditioning led to upregulation of genes responsible for stress, resistance to toxic compounds and antibiotics, and siderophores.

Conclusion: Tobacco smoke causes downregulation of several genes essential for survival of commensals in a biofilm environment and also upregulation of several virulence factors in secondary biofilms. These biofilms normally offer protection against disease; by changing gene expression patterns in these communities, smoking creates pathogenic 'miscreants' out of this eubiotic community.

NEW APPROACH FOR *PROPIONIBACTERIUM ACNES* BIOFILM TREATMENT IN *ACNE VULGARIS*: MYRTACINE® ANTI-BIOFILM EFFICACY

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Recent works present evidence of *P. acnes* growing as a biofilm in follicles. This formation of clusters is now considered as an explanation for *in vivo* resistance of *P. acnes* to the main antimicrobials prescribed in *acne vulgaris*. Our objective was to explore this hypothesis and propose a new therapeutic approach focusing on anti-biofilm activity. At first, we designed specific *in vitro* models (dynamic and static) able to promote the growth of adhered bacteria. The loss of sensibility of *P. acnes* biofilm (48h) towards erythromycin, clindamycin and doxycycline was then checked considering sensible and resistant strains. Even if *P. acnes* biofilm appears as limited microcolonies, a very high level of resistance has been observed whatever antibiotic and strain (S or R) tested. In a second step, the activity of Myrtacine® (Mediterranean Myrtle extract–Botanical Expertise P. Fabre) against biofilm formation and mature biofilm (48h) was evaluated using the dynamic model. Myrtacine® expresses an antibacterial activity on planktonic *P. acnes* with MIC ranging from 0.31 to 3.12 mg/L (strains eryS and eryR). Considering anti-biofilm activity, we noted an inhibition of biofilm formation (addition of Myrtacine® at D0) and a significant effect on mature biofilm (48h) since 1 min of contact. This potent therapeutic effect was checked using the static model for Myrtacine® concentrations ranging from 0.03% to 0.0001%. A significant and dose-dependent anti-biofilm effect was observed even at concentration under MIC, i.e. 0.001%. At least, the interest of the combination of Myrtacine® with antibiotics was explored. A synergistic efficacy was noted when erythromycin (1000mg/L), clindamycin (500mg/L) were added to 0.001% Myrtacine®. These results are in accordance with preliminary *in vivo* studies on Myrtacine® indicating a reduction in *acne vulgaris* symptoms. An *in vivo* experiment was in progress to demonstrate the impact of Myrtacine® on *P. acnes* counts and biofilm formation.

This study received financial support from Lab Pierre Fabre, France.

PII-3

EXAMINATION OF THE OCCURENCE OF *FUSOBACTERIUM NUCLEATUM* IN ORAL TUMOR BIOFILMS

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Fusobacterium nucleatum is an opportunistic anaerobe species in the oral cavity playing an important role in periodontitis and it is associated with intrauterine and other extra-oral infections. Recently its association with colorectal cancer (CRC) has also been demonstrated where *F. nucleatum* has been proved that it could invade the colon's epithelial cells and promote the carcinogenesis via an inflammatory process. The key figure in this process is the *F. nucleatum*'s unique adhesin the FadA which can bind the epithelial cell's E-cadherin and activates β-catenin signaling, and differentially regulates the inflammatory and oncogenic responses. In our experiments, we wished to investigate the association of *F. nucleatum* with oral squamosus cell carcinomas (OSCC) in which the role of E-cadherin-mediated signalization is also an important transformational factor. We emphasize that the *F. nucleatum*'s main habitat is the oral cavity where it has the maximal occurrence rates and it also has a chance to interact with E-cadherin despite the histological differences of the epithelia between the intestine and the oral cavity.

Biofilm samples were obtained from the central surface from the malignant or pre-malignant lesions and from the contiguous healthy mucosa from the same patients. Total DNA was extracted, and the rates of carriage of *F. nucleatum* were detected by 16S RNA RT-PCR using serially diluted *F. nucleatum* samples as calibration controls.

The detected *F. nucleatum* CFUs in our samples were in the range of 1-5x10 to $3x10^4$. The changes in the prevalences of *F. nucleatum* between the malignant or pre-malignant and the healthy sides where in the range of 1 and 60. The severity of the malignancy, e.g. lichen or OSCC and the changes in *F. nucleatum* CFUs correlated.

Although the number of patients still examined is low, the association of *F. nucleatum* with oral malignancies can be inferred. Other experiments, as bacterial FISH of tumor tissues are also planned.

1250 POSTER SESSION II: DIAGNOSTIC AND LABORATORY TECHNIQUES

Posters will be presented in Poster Session II
Monday, June 30 1250-1350.

HYDROCARBON SYNTHESIS *DESULFOBACTERIUM MACESTII 1598* WHEN GROWING BACTERIA IN A BIOREACTOR

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Among energetically significant products synthesized by microorganisms hydrocarbons remain poorly understood. The exception is methane, the synthesis of which was studied in detail by many researchers.

Previously we have found that sulfate-reducing bacteria are able to synthesize extracellular hydrocarbons with chain length C_{11} - C_{24}. However, the yield was not that high.

In order to increase the yield of the desired product and simulate the formation of hydrocarbons strain of sulphate-reducing bacteria—*Desulfobacterium macestii 1598*—we have performed experiments on a bioreactor of 2.5 liters, during 7 days at 37°C where bacteria was cultured. The gaseous phase contained a mixture of gases H_2 : CO_2 = 9:1. The ratio of the volume of the culture medium to volume of the gas was 1:2.

Studies have shown that during the growth *Desulfobacterium macestii 1598* active consumption of gas mixture occurs (150-200 ml per day), a new portion of the reactor which was supplemented daily. At the end of the experiment the analysis showed that the amount of hydrocarbons was 90.2 ± 2,6 2 mg / l. Significant indicators were Eh and pH. Best hydrocarbon synthesis observed in Eh (-300) - (-350) mV, pH 7.0-7.2.

Strain synthesized hydrocarbons were determined mainly in the field of C_{11} - C_{24}. While growing cells have shown an increse over time as normal, as well as isoforms hydrocarbons, with a chain length of C_{19} to C_{24}.

Introducing additional fatty acid (oleic) also increased the synthesis of hydrocarbons 3-5 times, mostly due of the synthesis of alkanes with a chain length of C_{19} - C_{24}. Quantitative advantage of normal hydrocarbons remain unchanged.

Thus, increasing the volume of nutrient medium, and especially the volume of the gaseous phase, promote increases in the amount of hydrocarbons synthesized by sulfate-reducing bacteria. Additional supplement to the culture medium of fatty acids have shown that they may be involved in the synthesis of hydrocarbons, without being substrates for microbial growth. In this case, apparently, the process of regeneration of both CO_2 and fatty acids occurs.

PROLINE FOR CONFIRMATION OF *CLOSTRIDIUM DIFFICILE* COLONIES IS NOT 100% RELIABLE

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Objective: *Clostridium difficile* is usually recovered from diarrheal stool samples using selective and differential agar media, such as CCFA, CCFA-HT, or Chrom-Agar. Other clostridia occasionally grow on these media thus necessitating a confirmatory test for identification. While some laboratories used molecular methods or preformed enzyme panels, proline disks have been recommended as a rapid method to confirm the identity of *C. difficile*. We evaluated this method on 2305 recent isolates of *C. difficile* recovered during a clinical trial of CDAD.

Methods: Suspected colonies of *C. difficile* isolated from primary stool culture were subcultured onto Brucella agar for purification, subsequent testing, and storage. A proline disk was placed on an isolated colony for about three minutes. The disk was removed to a sterile petri dish and one drop of DMACA reagent was added. Positive tests turned a deep pink-purple within 30 seconds. Colonies that appeared to be *C. difficile* but were proline-negative were further identified using 16S rRNA gene sequencing.

Results: Of 2305 isolates tested to date, 14 were negative using the proline disk test, but identified as *C. difficile* by 16S rRNA gene sequencing.

Conclusion: While a positive proline test is highly accurate, labs should be aware that 0.6% of *C. difficile* strains test negative and use other means to confirm suspected colonies of *C. difficile* when that is the case.

FREQUENCY OF POSITIVE ANAEROBE BLOOD CULTURE IN A TERTIARY HOSPITAL OF MEDELLIN COLOMBIA

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In Colombia, the real frequency of obligate anaerobes causing bacteremia is not well studied, and there is not a protocol for selective or routine anaerobic blood culture use. Therefore, the aim of this work was to determine the frequency of positive anaerobic blood cultures in patients with clinical suspicion of bacteremia in a tertiary hospital of Medellin. Two aerobic and one anaerobic blood culture bottles were taken simultaneously from patient with systemic inflammatory response syndrome-SIRS, incubated for five and seven days, respectively, at 37°C in an automatized system blood culture. Anaerobic and aerobic bacteria were classified *according* to Wadsworth-KTL Anaerobic Bacteriology Manual and ASM's Clinical Microbiology Procedures Handbook. A total of 188 patients were evaluated between November 2011-April 2012. From these, 116 (59%) were male, 150 (79.7%) were receiving antimicrobial therapy at the moment of venopuction. From the former, 46% received beta lactam inhibitor antibiotics, 27% carbapenem, 17% glycopeptides and 10% others. The most frequent underlying conditions were hematological disease (31%), followed by cardiovascular disease 19% and solid tumor 9%. The frequency of positive anaerobic blood cultures was 0.5% (1/188), and from this bottle, taken from a 66 year-old woman with breast cancer and UTI diagnosis, *Bacteroides fragilis* and *Staphylococcus aureus* were simultaneously isolated. The positivity for aerobe bottles was 22% (41/188), the most frequent germen isolated were *S. aureus* (24.3%), followed by *Staphylococcus epidermidis* (11.9%), *Pseudomonas aeruginosa* (11.9%), *Klebsiella pneumoniae* (11.9%) and *Escherichia coli* (9.5%). In addition, an *E. coli* was recovered from an anaerobic blood culture bottle while the corresponding aerobic bottles were negative. This study is a first approach to determining the frequency of positive blood culture in a tertiary hospital in Medellin and the results suggest the importance of establishing the use of anaerobic blood culture according to focal anaerobe infection frequency. Further studies on prevalence and risk factors for bacteremia in hospitals of Medellin are necessary for the generation of appropriate diagnosis protocols.

SEQUENCE ANALYSIS PIPELINE FOR COMPLEX MICROBIAL COMMUNITIES

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Purpose: Understanding human-associated microbial ecology is essential for insight into health, as well as identifying disease states, risk factors, and etiology. The 16S ribosomal RNA gene is the most common genetic marker for taxonomic identification due to its near universal presence and static function over time, and a wide variety of tools exist for quantifying samples. The popular QIIME software was developed to gather such tools into a single, usable pipeline, but is less usable when species-level analysis is important; as is the case with highly complex oral biofilms. We have developed a new set of tools that wholly integrate with the QIIME pipeline to reduce primer bias, enhance species-level analysis and visualization, and greatly improve analysis speed.

Methods: All tools were developed using the Python programming language, and use QIIME output files as input, but do not directly depend on a QIIME installation. All analysis and generation of data for visualization was performed with resources provided by the Ohio Supercomputer Center.

Results: Our new pipeline was applied to three oral microbiome datasets examining combinations of salivary, supragingival, and subgingival bacterial community composition in smokers and non-smokers, pediatric subjects, and dental implant recipients. Compared with the QIIME-provided Amazon EC2 instance, our pipeline reduced processing time for 2 million 16S sequences from over one week to less than nine hours. Furthermore, we developed and applied algorithms to reduce single-primer bias, condense redundant taxonomic output, and automatically generate the data for visualizing phylogenetic quantification.

Conclusion: We have developed a set of analysis tools targeted at enhancing taxonomic identification of microbial samples while using the QIIME software pipeline. When applied to several large samples of the oral microbiome, analysis times were drastically decreased and species-level analysis was substantially enhanced. Our tools integrate with the QIIME pipeline and are available free and open source.

ESTABLISHING A MURINE MODEL OF *CLOSTRIDIUM DIFFICILE* INFECTION: TRIALS AND TRIBULATIONS

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Purpose: To establish a murine model for *Clostridium difficile* infection (CDI) at our Institution, the University of Wisconsin, Madison, in which to test the efficacy of novel therapeutic agents against CDI.

Methods and Results: We followed the methods of two published mouse models, A and B, for CDI. Seven week old male C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were housed with autoclaved food, bedding and water with cage changes and manipulation of mice performed within a laminar flow hood. All mice were pre-treated with antibiotics to disrupt endogenous microbiota and create vulnerability to CDI. Mice in model A trials received a 3-day oral antibiotic cocktail of kanamycin, gentamicin, colistin, metronidazole, and vancomycin in sterile drinking water plus a single intraperitoneal injection of clindamycin. Trials following model B utilized the cefoperazone for 10-days in sterile drinking water, followed by a single intraperitoneal injection of clindamycin. The mice were subsequently challenged via oral gavage with *C. difficile* spores or vegetative cells of strains including ATCC 43255 and ATCC BAA-1870, at concentrations ranging from 10^1 to 10^7 colony forming units/mouse. Mice were monitored for changes in health score, including weight for 10 days post *C. difficile* challenge. Percent survival and health score were recorded and used as a benchmark for the establishment of disease. Major differences between method A and B were the oral antibiotic regimen and the length of time it was administered. In addition, mice were obtained from specific mouse facilities within Jackson Laboratories depending on each mouse trial. The strain and dose of *C. difficile* varied between trials as well as whether or not it is in vegetative or spore-form.

Conclusions: In total, we completed five trials and obtained disease using method B. Some important considerations to keep in mind when establishing a murine model for CDI include the type of antibiotics received, number of animals per cage, mouse source, food, strain of *C. difficile*, and whether vegetative cells or spores are used. Our results have implications for other research programs interested in establishing a murine model of CDI.

THE NEW APPROACH FOR CLASSIFICATION OF *BACTEROIDES* BY HOUSEKEEPING GENES FROM GENOME SCREENING

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Background: The members of genus *Bacteroides* are the opportunistic anaerobes most frequently isolated from human infections. In the past few years, many studies have revealed a remarkable genetic variability in *Bacteroides* species. As for particular genes, 16S ribosomal RNA gene, RNA polymerase b subunit gene, and heat shock protein gene have been suggested as useful maker for classification of *Bacteroides* species. However, it is difficult to prove which genes are the most effective for the classification. The aim of this study was to evaluate the use of DNA sequences and amino acid sequences commonly found in *Bacteroides* species.

Study: In present study, we used amino acid sequences from PATRIC database (http://patricbrc.org/portal/portal/patric/Home), which were determined by genome sequence analysis. From the results of *Bacteroides* genome sequence analyses, more than 300 of gene that were commonly distributed in the genus were found. Those genes were selected and used for their phylogenetic analysis. Amino acid sequence diversity of these genes was calculated, and several high diversity genes were selected as candidates of remarkable gene for classification of *Bacteroides*. We also compared their DNA sequences and amino acid sequence variation to investigate whether they were useful for their species level and genus level classification. In addition to those analyses, thirty-nine ribosomal proteins were analyzed to compare the variation among members of genus *Bacteroides*.

Conclusion: It is suggested that amino acid sequence analysis is a new useful approach to investigate phylogenetic relationship in *Bacteroides*.

USE OF HEPA FILTRATION WITHIN AN ANAEROBIC CHAMBER TO REDUCE BACTERIAL DENSITY IN THE INCUBATION ATMOSPHERE

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The presence of bacterial cells or spores within the atmosphere of an anaerobic chamber may cause cross contamination between cultures, present a risk to the operator and lead to ejection of viable bacteria into the external environment via excess gas or water released from the chamber. We have developed an anaerobic workstation with an integral, custom-made HEPA filter that achieves 0.3 µm particle counts better than ISO 14644-1 Class 3 within the chamber. We evaluated the removal of vegetative bacteria (*Kocuria rhizophila*) and spores (*Clostridium beijerinckii*) from the incubation atmosphere of this workstation during normal operation and compared its performance with an equivalent model not equipped with a HEPA filter. Before testing, each workstation was allowed to stabilize at 37°C and 70% relative humidity and was operated continuously throughout the test so that normal atmospheric circulation occurred. Suspensions of *K. rhizophila* cells and *C. sporogenes* spores were aerosolized using a 6 jet Collison nebulizer within each workstation, to produce calculated viable counts of >106 cfu per m3. Viable bacteria in the incubation atmospheres were collected quantitatively, at 5 minute intervals, using an air sampler and appropriate agar plates. Condensate water (pumped out of the workstations during normal use) was spread onto agar plates for bacterial enumeration and gas expelled through the exhaust valves was sampled by exposure of agar plates. No viable bacteria were recovered from the HEPA filtered workstation 10 minutes after aerosolization, while bacteria of each species remained abundant in the standard workstation for at least 4 hours. In addition, viable bacteria were recovered from the gas exhaust of the standard workstation but not from the HEPA filtered unit. Thus, the use of HEPA filtration in an anaerobic chamber produces a substantial reduction in bacterial contamination of the atmosphere and reduces ejection of bacteria into the laboratory environment.

IDENTIFICATION OF *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII* IN A HOSPITAL UNIT IN GREECE

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Pseudomonas aeruginosa (PA) and *Acinetobacter baumannii* (AB) are an ordinary reason of serious infections in hospitalized patients and is linked with high rates of hospital mortality and morbidity. The aim of present study was detection and molecular typing of clinical strains of PA and AB isolated from patients in hospital units in Greece. The strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were isolated from various clinical specimens in a single health-care facility during 2012-2013. Duplicated samples positive for AP and PA were excluded from present study. Identification was performed using the automated system VITEK2 (BioMerieux, France). The molecular epidemiology and the genetic relationship between the PA and AB strains were investigated using RAPD analysis. A total of 25 and 17 *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains were detected, respectively. Based on RAPD patterns, *Pseudomonas aeruginosa* strains were classified into 13 operational taxonomic units (OTUs), while *Acinetobacter baumannii* strains into 9 OTUs. Our results indicated a magnitude of genetic diversity among the isolated strains. This study highlights the necessity for clinical surveillance of bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in hospitals by adopting molecular typing methods.

ACCURACY OF ANAEROBIC BACTERIA IDENTIFICATION BY BRUKER MICROFLEX MALDI-TOF

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Purpose: To validate the identification of clinical anaerobic bacteria by the Bruker Microflex MALDI-TOF instrument.

Methods: A total of 160 clinical isolates of anaerobic bacteria (89 species) obtained from Stanford Clinical Microbiology laboratory were evaluated. Identification results by the Bruker MALDI-TOF (Biotyper 3.3.1.2), using direct application and on-plate Formic Acid extraction when direct method failed to identify, were compared with the results from parallel testing of RAPID ANA (Remel) or VITEK ANC (bioMerieux). Further 16S RNA gene sequencing was used to resolve discrepancies.

Results: Using the cutoff score of 1.7 for identification to the species level, 18 isolates (11.3%) were not identified because they were missing from the database. All of the remaining 142 organisms (88.7%) were correctly identified to the genus level with a sensitivity of 100%, to the species level with a sensitivity of 97.1% and a specificity of 100%. MALDI-TOF correctly identified to the species level 96.7% of 60 Gram negative rods, 98.4% of 61 Gram positive rods, 100% of 5 Gram negative cocci, and 92.9 % of 14 Gram positive cocci.

Conclusion: Our validation data showed MALDI-TOF is an accurate tool for identification of anaerobic bacteria. The accurate results in conjunction with rapidity and simplicity of use make the MALDI-TOF an essential tool in clinical microbiology practice.

PROPIONIBACTERIUM KOCII A NEW HUMAN PATHOGEN ANAEROBIC BACTERIA

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Objectives: *Propionibacterium* species are nonsporulating, gram-positive anaerobic bacilli that are considered as commensal bacteria on the skin. They are usually non-pathogenic and are common contaminants of blood and body-fluid cultures. Sometimes, it can be difficult to determine whether positive culture results for propionibacteria reflect contamination or true infection. The role of *P. acnes* in the pathogenesis of acne has been debated for decades, but never adequately proven. Serious infections due to *propionibacteria* are rarely reported, but this bacterium is increasingly recognized as a cause of serious infections, such as endocarditis, prosthetic joint infection, endophthalmitis, osteomyelitis and central nervous system infections.

Methods: *P. kocii* strains were isolated from the following three different cases: Case 1. A 15 year old female patient with inflammatory acne receiving systemic lymecycline treatment. The strain was tetracycline sensitive, sorbitol and protease positive, Case 2. An intraoperative sample from a 55 year old female patient, who had a benign brain tumor, we could isolate the strain as a pure culture in high CFU, Case 3. An intraoperative abscess sample from a 60 year old male patient who had a malignant brain tumor, only this strain was isolated in very high CFU.

Results: MALDI-TOF analysis gave only *Propionibacterium* sp. results, because this species was not included until this time in the database. Genome sequencing was performed by combining the cycled ligation sequencing on SOLiD V4 System (Life Technologies) with 454 FLX pyrosequencing (Roche). It has a single circular chromosome of 2,410,997 bps, with a GC content of ~60%; there are 2205 putative coding sequences, 49 tRNAs, and 9 rRNA loci.

Conclusion: *Propionibacterium* infections are usually characterized by a paucity of classical symptoms of infection or inflammation. Invasive propionibacterium infection typically occurs in the setting of after surgery. Given the low virulence of propionibacteria, infections with these organisms are usually indolent. In our cases we could detect this species by molecular methods and we found a "new" species when clinical manifestations were connected to this bacterium. Using a new molecular techniques, new or earlier non-pathogenic bacteria can be indentified in various clinical pictures or we can elucidate the pathogenic role in these settings.

ANAEROBIC BACTERIA ISOLATES FROM FOOTROT LESIONS OF SHEEP AND GOATS

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Anaerobic bacteria isolates from footrot lesions were examined. Footrot in small ruminants, such as sheep and goats, is a serious limit to animal health, welfare and productivity. The acute or chronic infection in hooves of small ruminants is usually referred to as 'foot scald' for less virulent conditions and 'footrot' for severe virulent hoof infections. Although bacteria strains of *Dichelobacter nodosus* have been identified as major pathogens for virulent footrot, in most cases, footrot is preceded with foot scald involving infection of mixed aerobic and anaerobic bacteria. Foot scald and footrot cases were inspected and foot lesion swabs cultured for 196 animals (sheep = 186 and goats = 14) on 14 farms (Missouri = 12, Oregon = 1 and Maine = 1) for this study. Footrot and foot scald symptoms, such as lameness, hoof and interdigital lesions were graded with footrot scores from 0 (no infection) to 4 (severe infection). Specimen swabs were collected in chopped meat media transport tubes from infected toe, hoof or interdigital skin lesions. Swab samples were inoculated on either blood agar or nutrient broth-based plates, and incubated in an anaerobic chamber at 37°C. Primary isolates were sub-cultured and identified for bacteria genus or species using the following systems: Sensititre AP-90 and AP-80 Gram Identification Systems, Sherlock® Microbial ID Systems, and GEN III Microplate™ (Biolog, Inc). Footrot lesion score was analyzed by one-way ANOVA. There was no significant ($P > 0.05$) correlations of footrot score severity with a particular bacteria species. Virulent footrot pathogen *D. nodosus* was not recovered from these isolates. However, more severe foot scald and footrot were closely associated with increasingly dominant anaerobic isolates. The most frequently isolated genus and species of these anaerobic phylum were Actinobacteria *(e.g., Actinobaculum suis* and *Actinomyces bovis),* Fusobacteria *(Fusobacterium spp.),* Proteobacteria *(Desulfovibrio spp.),* Firmicutes *(Lactococcus spp., Clostridium spp.,* and *Peptostreptococcus spp.),* Bacteriodetes *(Bacteroides spp., Porphyromonas spp., Prevotella spp.,* and *Sebaldella termitidis)*. Each of these groups contribution to footrot infection and severity were unverifiable except that *Fusobacterium necrophorum* was well documented as nearly equal in virulence as *D. nodosus* in footrot diagnosis.

CHANGES IN *CLOSTRIDIUM DIFFICILE* STRAINS RECOVERED FROM PATIENT SPECIMENS FOLLOWING INTRODUCTION OF A MULTI-STEP TESTING PROTOCOL WITH INCREASED SENSITIVITY

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Many laboratories have documented increased rates of *C. difficile* (CD) detection after replacing the relative insensitive toxin immunoassays (toxin A/B EIA) with multi-step testing (MST) protocols that include glutamate dehydrogenase (GDH) and/or PCR tests. In order to understand the effect of this change in testing strategy on the epidemiology of recovered CD strains, a retrospective analysis of CD-positive stools was conducted at the Edward Hines VA Medical Center from June 2010 to June 2011. Prior to December 2010, toxin A/B EIA was the diagnostic test employed. In December 2010, a MST approach was adopted; an EIA that detected both GDH and toxin A/B was used as the initial screening. Those specimens that were positive by GDH but negative by toxin A/B were also tested for *tcdB* by PCR. Available CD-positive stool specimens where cultured anaerobically on selective media for CD and restriction endonuclease analysis (REA) strain typing was performed on the recovered CD isolates.

In the 6-month pre-MST period, 629 stool specimens were submitted for testing and 79 were positive for CD (12.5%). In the 6-month post-MST period, 396 stool specimens were submitted for testing of which 121 were positive (30.5%). Pre-MST, a total of 8 different REA group strains were identified compared with 14 REA group strains post-MST. In addition, a clostridium species other than CD was recovered from 2 of the clinical specimens that were test-positive for CD post-MST. The epidemic REA group BI strain was identified in 46% of the recovered CD isolates pre-MST and in 54% post-MST. Representative CD isolates from each REA group before and after the implementation of MST were analyzed for quantitative toxin A/B production *in vitro* by immunoassay. Pre-MST, 3 of the 8 REA groups (37.5%) showed low toxin production (<400ng/mL) *in vitro* compared to 8 of the 14 REA groups (57.1%) recovered post-MST.

The increased sensitivity of CDI testing by MST can be explained, at least in part, by detection of a wider variety of CD strains, some of which are low toxin-producers *in vitro*.

1250 *POSTER SESSION II:* THE CARE AND FEEDING OF OUR INTESTINAL MICROBIOME

Posters will be presented in Poster Session II
Monday, June 30, 1250-1350.

PRODUCTION OF FERMENTED SAUSAGES WITH OLIVE OIL, DIETARY FIBERS AND LACTIC ACID BACTERIA WITH PROBIOTIC PROPERTIES

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The objective of this study was to evaluate the microbiological quality, sensory acceptability, dietary value, and viability of strains of lactic acid bacteria (LAB) with probiotic properties of fermented sausages produced with total replacement of pork fat with olive oil and addition of probiotic cultures and dietary fibers.

Four treatments of sausages were produced. Olive oil was emulsified with turkey proteins, solidified with heat treatment and then added replacing pork fat. The first treatment was prepared without the addition of LAB and three treatments were prepared with three different cultures of LAB with probiotic properties (*Lb. acidophilus, Lb. casei, Lb. sakei*) originating from traditional sausages, identified and tested for their safety and probiotic properties in a previous study. Fermentation was completed in 21 days. Physicochemical, nutritional, sensory and microbiological analyses were carried out in all treatments at the 1st, 3rd, 7th, 14th and 21st day of fermentation.

Weight loss, pH values and a_w were developed as expected. What is important as for the nutritional value of fermented sausages with olive oil is that they have less fat content than the conventional fermented sausages, which consists of mono-unsaturated fatty acids and antioxidants. Olive oil is more stable to oxidation than pork fat.

Treatments with LAB cultures added had improved sensory properties and structure comparing to the blank sample and they were safe from pathogens from the 7th day of fermentation. Final counts of live LAB with probiotic properties were >10^6-10^7 cfu/g comparing to the blank sample that had <10^6 cfu/g. All LAB added were adapted to fermentation conditions. Treatments with the addition of *Lb. casei* and *Lb. sakei* showed the highest counts of LAB in the final samples with accepted decrease of pH values.

In conclusion, fermented sausages with olive oil, probiotics, and dietary fibers, belong to new eco-innovative products that are not only technologically complete, but also safe and healthy for the consumer.

IN VITRO ASSESSMENT OF LACTIC ACID BACTERIA ISOLATED FROM CHEESE AS POTENTIAL PROBIOTICS

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The last decades, research in the probiotic area has established the health benefits associated with them and there is a major progress in the characterization and selection of specific probiotic cultures. The main source of probiotics is traditionally the fermented foods. Dairy products are the main vehicles for delivery of probiotic microorganisms into the human intestine. The selection of a microorganism to be used as probiotic is based on particular criteria. These are recapitulated to the following: they must be of human origin and be safe for humans (generally recognized as safe: GRAS), since usually the products are intended for human consumption, they should preserve their survival when they pass through the GI tract and have the ability to attach to the GI tract, they should be resistant to the low pH values and also to the action of the hydrolytic enzymes of the GI tract and to exhibit antimicrobial properties. Lactic acid bacteria (LAB) are the most important group of microorganisms commercially used as starter cultures for the manufacture of dairy based probiotic foods like cheese. Likewise, various LAB were isolated from cheese and were screened through some *in vitro* test for probiotic character appearance. More specifically, the tests that were followed were: survival under conditions simulating the human GI tract, resistance to low pH, bile salts hydrolysis and antimicrobial activity against pathogens.

SCREENING OF VARIOUS LACTIC ACID BACTERIA ISOLATED FROM CHEESE FOR THE ASSESSMENT OF PROBIOTIC PROPERTIES

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Functional food production is an upsurge scientific area the last decades. Probiotic foods are considered as the most important subcategory of functional foods. Likewise, the application of probiotic bacteria in food systems is very critical. To deliver the health benefits, probiotics need to contain an adequate amount of live bacteria (at least 10^6 –10^7 cfu/g) and be able to survive the acidic conditions of the upper GI tract and proliferate in the intestine, a requirement that is not always fulfilled. Therefore, in the frame of the present research survey various lactic acid bacteria (LAB) were isolated from cheese samples and their probiotic properties were assessed by investigating their survival after transit through the GI tract. Particularly, various *in vitro* studies were conducted such as the determination of acid and bile tolerance, tolerance to artificial intestinal juice and bile salts. In addition the expression of potential protective action against pathogens was studied, by application of various antibiotics.

Acknowledgement
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PRESENCE OF *BIFIDOBACTERIUM SPP.* IN CHILDREN THAT RECEIVED AT BIRTH BREAST MILK AND FORMULA MILK

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Bifidobacterium species are anaerobic bacteria belonging to the human and animal intestinal microbiota and they are important for the balance of the intestinal microbiota. The intestinal microbiota of breast-fed infants is primarily composed by lactic bacteria, such as bifidobacteria and lactobacilli. The intestinal microbiota of formula-fed infants is diverse harboring species of *Bacteroides*, *Clostridium* and *Enterobacteriaceae*. In this study, the presence of *B. adolescentis*, *B. breve* and *B. infantis* in children aged from 3 to 12 years was determined by culture and PCR. Fresh stool samples from 113 healthy children were collected. They were fed until 6 months of age with different diets, breast milk (60), formula milk (18) and breast and formula milk (35). Stools were streaked onto *Bifidobacterium* medium, and bacteria were identified by conventional PCR. DNA from stools was used to detect species of *Bifidobacterium*. By culture *Bifidobacterium spp*. was detected in 63.7% and by PCR in 96.4%. In all children groups, *B. breve* was recovered by culture in 9.8% and by PCR in 16%. *Bifidobacterium adolescentis* was recovered by culture in 49.5% and by PCR in 61%; and *B. infantis* by culture in 27.4% and by PCR in 88.4%. Our results show no significant difference among the presence of *Bifidobacterium spp*., *B. adolescentis*, *B. breve* and *B. infantis*, as well as, among the type of feeding that these children received at birth.

INVESTIGATION OF PREBIOTIC CHARACTERISTICS OF FRUCTOOLIGOSACCHARIDES FROM FRUITS: METABOLIZATION BY PROBIOTIC AND ENTEROPATHOGENS INHIBITION

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Fructooligosaccharides (FOS) are well known prebiotic. We studied here prebiotic characteristics of FOS synthesized from fruits sugars, which are different from other FOS with more intermediate polymers and sorbitol.

Probiotic bacteria were used to evaluate the ability of our compounds to promote growth of beneficial strains. Commercialized Raftilose®L95 and Actilight®950P were used as a 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 FOS from fruits were metabolized by all probiotics, sorbitol was fermented by 80% of them, at the same rates that those observed with Actilight®950P whereas only 3 strains used Raftilose®L95. HPLC analyses were conducted to check the consumption of each degree of polymerisation (DP). DP2 and DP3 were preferentially consumed, but bifidobacteria were able to metabolize DP4. Several enteropathogen growths were also checked when co-cultured with probiotics as described above with equal inoculi and were differentiated on selective agar. *Candida albicans*, *Clostridium difficile*, and *Listeria monocytogenes* to a lesser degree, were efficiently inhibited by some lactobacilli and bifidobacteria with most efficiently with FOS from fruits than for Raftilose®L95. We also monitored the anti-proliferative effect of our compounds on human epithelium cancer cells HT-29 by an XTT assay. Two probiotic strains lead to a dramatic decrease in cells proliferation only with FOS from fruits.

We demonstrated here *in vitro* that FOS from fruits promote probiotics growth and could inhibit more efficiently *C. difficile* and *C. albicans*, some major pathogens involved in antibiotic-associated diarrhoea, than some commercialized FOS. In addition, only FOS from fruits could lead probiotic to reduce significantly cancer cells proliferation. Therefore, FOS from fruits 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.

ASSESSMENT OF ANTIMICROBIAL PROPERTIES OF CHIOS MASTIC GUM ESSENTIAL OIL AGAINST FOODBORNE PATHOGENS

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Nowadays, there has been an increased interest in essential oils from various plant origins as potential antimicrobial agents. This trend can be mainly attributed to the rising number and severity of food poisoning outbreaks worldwide along with the recent negative consumer perception against artificial food additives. Hence, the aim of the present study was to investigate potential antimicrobial action of Chios mastic gum essential oil against foodborne pathogenic bacteria. Chios mastic gum is a natural resin obtained from *Pistacia lentiscus*, an evergreen tree which is cultivated mainly in the Greek island of Chios and in other Mediterranean area countries. Chios mastic gum essential oil was isolated by direct gum distillation and analyzed by GC/MS. The main components identified were *a*-pinene (67.7%), myrcene (18.8%) and *b*-pinene (3.0%). Subsequently, *in vitro* antibacterial activities were evaluated against *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The antimicrobial properties were initially assayed by the disk diffusion method and then the minimum inhibitory (MIC) and non-inhibitory concentration (NIC) values were assessed using a microplate reader and an automated technique which combines the absorbance measurements with the common dilution method. Non-linear regression analysis was used to fit the data using a previously published model. The results revealed that Chios mastic gum essential oil is a noteworthy growth inhibitor against the bacteria studied and indicated that it represents an effective and inexpensive source of potent natural antibacterial agents, which may be usefull in the food industry.

PROBIOTIC PROPERTIES OF IMMOBILIZED *LACTOBACILLUS CASEI* ATCC 393

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Nowadays, an upsurge of interest in developing novel foods containing probiotic microorganisms is observed. To deliver the health benefits, probiotics need to contain an adequate amount of live bacteria, able to survive the acidic conditions of the upper GI tract and proliferate in the intestine. Since it is well established that cell immobilization enhances the viability of cultures, the aim of the present study was to assess potential probiotic attributes of immobilized *L. casei* ATCC 393 on apple pieces in comparison to free cells. In *in vitro* GI stress tolerance tests, immobilized *L. casei* ATCC 393 on apple pieces exhibited significantly higher survival rates compared to free cells. High adhesion ability was recorded by adding free or immobilized bacteria to a monolayer of Caco-2 cells, while the strain was characterized as relatively hydrophilic. To investigate *in vivo* survival in the GI tract, probiotic fermented milk containing either free or immobilized cells was administered orally at a single dose or daily in Wistar rats. By 12h after single dose administration, both free and immobilized cells were detected at levels ≥ 6 logcfu/g of feces. Adhesion of the probiotic cells at the intestine was a targeted process, as their levels ranged ≥ 6 logcfu/g (minimum suggested levels for conferring a probiotic effect) at the large compared to levels ≤ 3 logcfu/g at the small intestine following daily administration for 7 days. Finally, daily administration of the probiotic products led to significant reduction of staphylococci, enterobacteria, coliforms and streptococci counts in rat feces.

IDENTIFICATION OF LACTIC ACID BACTERIA ISOLATED FROM GREEK TRADITIONAL FERMENTED SAUSAGES AND THEIR SAFETY CHARACTERISTICS AND PROBIOTIC PROPERTIES

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The aim of this study was the isolation and identification of lactic acid bacteria (LAB) from Greek traditional fermented sausages and the evaluation of their probiotic properties in order to be used in the production of dry fermented sausages. A total of 8 salami and 8 soutzouki were chosen from different regions in Greece ensuring that processing units do not use starter cultures in manufacturing of their products.

A total of 160 different colonies of LAB were randomly selected from MRS agar and subjected to gram stain and biochemical (oxidase, catalase) tests. After purification, 44 isolates were identified to species level by sequence analysis of their 16S-23S rDNA intergenic spacer region. By molecular identification, these isolates were classified into 6 different LAB species: *Lb. sakei* (47%), *Ped. pentosaceus* (23%) and *Lb. casei* (12%) predominated, followed by *Lb. brevis* (6%), *Lb. plantarum* (6%) and *Ped. acidilactici* (6%).

All 44 isolates were subjected to the following probiotic tests: growth in different temperatures, low pH and bile tolerance test, inhibition of pathogens, susceptibility to 20 antibiotics and production of biogenic amines.

Two strains of *Lb. casei*, four strains of *Lb. sakei* and three strains of *Ped. pentosaceus* were chosen because of their growth in low pH values, they were tolerant in bile salts presence, they had better antimicrobial properties and inhibition of the pathogens *S. Typhimurium, L. monocytogenes, S. aureus* and *E. coli*. These strains did not produce biogenic amines. On the contrary *Lb. acidilactici, Lb. brevis* and *Lb. plantarum* strains produced tyramine and one *Ped. pentosaceus* strain produced histamine.

These three LAB will be used in fermented sausage production not only as starter cultures, but as cultures with probiotic properties with $>10^7$ cfu/g in the final product.

1250 POSTER SESSION II: CLOSTRIDIUM SPP. HEALTH AND DESEASE

Posters will be presented in Poster Session II
Monday, June 30, 1250-1350.

CLOSTRIDIAL BACTEREMIA IN A RETURNING TRAVELER

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Clostridial bacteremia is an uncommon event. Herein is reported a case of *C. sordellii* bacteremia in a returning traveler as the presenting sign of malignancy.

A 29-year-old male presented to hospital six days after returning from an eight-day safari trip to Zanzibar and Tanzania. He reported three days' duration of nausea, vomiting, and constant right upper quadrant abdominal pain, accompanied by fevers, chills, and night sweats. Diarrhea and other constitutional symptoms were notably absent. Supportive care provided at an outside hospital the day prior had not improved his symptoms. He had obtained pre-travel immunizations, adhered to his malaria prophylaxis regimen, and followed food safety precautions. His wife who had accompanied him was well. Past medical history was remarkable for a Stage I testicular nonseminomatous germ cell tumor surgically treated five years earlier. CT imaging revealed enlarged bilateral retroperitoneal lymph nodes and a partial small bowel obstruction. Blood cultures exclusively grew *Clostridium sp*., with biochemical methods suggesting *C. bifermentans,* but 16s RNA sequencing identifying *C. sordellii*. Serial imaging revealed further retroperitoneal node enlargement, found to be rhabdomyosarcoma. After two weeks of metronidazole, he was referred for debulking surgery and chemotherapy.

Clostridial organisms are Gram-positive, anaerobic, spore-forming rods that form part of the intestinal microbiome. They account for only 0.5-2% of bacteremias, up to half of which are *C. perfingens*. Among clostridial species, *C. sordellii* and *C. bifermentans* are only rarely reported as pathogens, with *C. sordellii* most typically associated with uterine myonecrosis and gynecologic infections, and *C. bifermentans* with malignancy. The two species are difficult to distinguish biochemically, and may require 16s rRNA sequencing for definitive identification. Upon review of bacteremia cases, only 20 cases of *C. sordellii* and 10 cases of *C. bifermentans* have been reported and are often associated with comorbidities. Overall clostridial resistance rates to metronidazole remain low, but treatment also requires successful resolution of the predisposing pathology. 30-day mortality rates are high at 20-30%.

CLOSTRIDIUM PERFRINGENS TOXIN GENE TYPING AND WHOLE CELL PROTEIN PROFILE

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The spectrum of *C. perfringens* infections ranges from food toxinosis to myonecrosis. Whole cell protein and toxin gene types were profiled in 12 randomly selected *C. perfringens* veterinary stock cultures from the University of Wisconsin, Madison to determine epidemiological similarity or diversity amongst strains of animal origin. Whole cell protein analysis was done by SDS-PAGE while toxin gene typing was achieved by extracting DNA by boiling, DNA concentration and purity was determined by spectrophotometer and nanodrop while separation was by check gel electrophoresis. Multiplex PCR was used to identify the toxigenic gene-type. *C. perfringens* B and *C. perfringens* EE with established profiles were used as control strains. Isolates typed included strains cp 296, 309, 12872 (from dogs) and 304, 305, 306, 341, 342, 10754, 12218-2, 12218-3, 12473 (from cow). All 12 strains possess the cpa gene, 4 strains have cpb_2, 3 strains etx, 2 strains positive for cpe and 1 for cpb. None of the strains carries the iA gene. Two strains have only cpa gene however no strains has more than two toxin gene types, with cpa-cpb_2 combination being more frequent. *C. perfringens* 305 (etx and cpa) and 342 (cpe and cpa) shared the same protein profile but belong to different toxinotype. It is evident that the cpa gene is a marker for all *C. perfringens* strains, and similarity in protein profile is not sin qua non for toxin gene type.

DETECTION OF TPEL AND NETB GENES IN *CLOTRIDIUM PERFRINGENS* ISOLATED FROM HEALTHY CHILDREN

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Clostridium perfringens is a constituent of the human and animal intestinal microbiota. This microorganism produces enteritis and enterotoxaemia in chickens, calves and goats, and it causes serious economic losses worldwide. *Clostridium perfringens* is able to produce several toxins, such as α-toxin and ϵ-toxin. Toxin *Clostridium perfringens* large cytotoxin (TpeL) and Necrotic Enteritis Toxin B-like (NetB) have also been described in isolates obtained from animal feces. In this study, the presence of *tpeL* and *netB* genes in *C. perfringens* isolated from healthy children was determined. Fifty five strains were previously identified as *C. perfringens* type A. Bacterial growth in BHI (anaerobiosis, 48 h) was harvested by centrifugation, and DNA was extracted by using a phenol-chloroform method. The detection of genes *tpeL* and *netB* was performed by PCR. Of the 55 *C. perfringens*, 5 (9.1%) harbored the gene *tpeL* and 7 (12.7%) the gene *netB*. All the 12 PCR products were purified and sequenced by using a Sanger method and all of them showed, respectively, 99% of homology by comparing with data of the GenBank. Our results suggest that healthy children may act as asymptomatic reservoirs of *C. perfringens* harboring both analyzed genes, and to our knowledge this is the first report in literature of the presence of these genes in humans. Furthermore, other assays might be done to confirm the toxin synthesis and the cell cytotoxicity.

GENE TPEL AND TOXIN TPEL IN *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM CHICKEN WITH NECROTIC ENTERITIS

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Necrotic enteritis is an important disease affecting the poultry industry and causing severe impaired production and economical losses worldwide. *Clostridium perfringens* is the causal agent of necrotic enteritis and is recognized that toxins, such as alpha toxin and NetB toxin are important virulence factors involved in the development of intestinal lesions. Large clostridial cytotoxins, including the recently discovered TpeL, affect the cellular morphology and they are related to intestinal diseases and myonecrosis. The role of TpeL in the pathogenesis of necrotic enteritis is yet unclear; however, recent studies have suggested that this toxin increase the lesion severity. In this study, we report the presence of *tpeL* in *C. perfringens* isolated from chickens with necrotic enteritis and the cytotoxic effect of TpeL on Vero cells. Seven (31.8%) out of 22 *C. perfringens* type A harbored the gene *tpeL*. DNA sequencing to confirm to presence of the *tpeL* in all the isolates was performed. Also, the bacterial supernatant was used to verify the cytotoxic effect on Vero cells in five time kinetics (3, 6, 24, 48 and 72 h) of incubation. All the strains *tpeL*$^+$ produced similar effect, such as morphological change characterized by the cell enlargement after 6 h, and after 48 h rounded cells were observed. At 72 h cell aggregates and eventual cell detaching from the wells were observed. The presence of toxin TpeL in *C. perfringens* isolated from chicken with necrotic enteritis appear to be the first report in Brazil. Additional studies are necessary to determine the role of the toxin TpeL of *C. perfringens* from different origin to verify its expression and mechanism involved in necrotic enteritis.

ISOLATION OF NOVEL CLOSTRIDIA SPECIES FROM AN AMAZONIAN COMMUNITY

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Since the launch of the Human Microbiome Project, nearly all studies to date have been performed using materials sampled from industrialized nations. Restriction of these studies within cosmopolitan areas is accompanied with biases such as similar diets, common use of antibiotics, and processed and chemically treated food and water supplies. In addition to molecular 16S rRNA gene surveys, cultivation and characterization of representative taxa from remote communities is essential to gain a more complete understanding of the ecology of the gastrointestinal tract. Our study is unique in that we study the human gastrointestinal microbiome of individuals from geographically remote, traditional native Amazon community in Peru.

We present data inferred from fecal samples collected from a traditional indigenous Shipibo Community from the Loreto Region in Peru. The diet of this group remains based largely on local agriculture supplemented with fishing and hunting from the surrounding jungle. Indigenous individuals, age 18-40, that were selected for the study, identified their grandparents and parents as also living within the community, removing biases such as the use of antibiotics and introduction of microbes from global food resources into their diets. A number of anaerobic enrichments were constructed using solid fecal material; one of these consisted of MRS broth with the addition of 20% ethanol under anaerobic mix headspace incubated at 37° C. A sample was transferred to MRS agar and sub-cultured until pure. 16S rRNA gene sequencing revealed that one of these organisms represents a novel Clostridium species and the results of its phenotypic, phylogenetic and chemotaxonomic characterization will be presented.

IMMUNOINFORMATIC ANALYSIS OF ALPHA AND TPEL TOXIN OF *CLOSTRIDIUM PERFRINGENS*

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Purpose: Necrotic enteritis (NE) is a common and severe *Clostridium perfringens*-induced disease in poultry. The bacterium synthesized multiple extracellular toxins. There are some reports on humeral immune responses against Alpha and TpeL toxin of *Clostridium perfringens*. Therefore, for elucidation and interpretation details of this immunoreactivity, we applied wide array of immunoinformatic tools in this study.

Methods: After retrieving Alpha and TpeL toxin and complete reference protein sequences, and evaluation their tertiary structure, wide array of immunoinformatic tools and severs applied for prediction/mapping of conformational (discontinuous) B cell epitopes. For conformational epitope by respect to obtaining reliable and effective consensus immunogenic epitope, we taking advantages of ElliPro, DiscoTope 2.0, SEPPA, CBTOPE, BCEP, and B-pred servers.

Results: In total, 3 and 2 unique and reliable B-cell antigenic regions were found for Alpha and TpeL toxin respectively; utilizing various wide array of computational analyses and immunoinformatics tools. The amino acids orders of 48-122, 160-210 and 270-370 for Alpha B-cell epitopes and 80-265 and 1130-1500 for TpeL toxin of *C. perfringens* were identified.

Conclusion: Present study, characterized most probable antigenic regions which are responsible for immune responses by hybrid approaches. Successful in-silico prediction of epitopes could be essential step for evaluating potential of protein immunogenicity and designing vigorous vaccines and diagnostic kits.

PREVALENCE OF NETB AND TPEL GENES AMONG *CLOSTRIDIUM PERFRINGENS* ISOLATES OBTAINED FROM HEALTHY AND DISEASED OSTRICHES (*STRUTHIO CAMELUS*)

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Purpose: *Clostridium perfringens* is an important pathogen in both human and veterinary medicine. Necrotic enteritis (NE) is the most clinically dramatic bacterial enteric disease of poultry induced by *C. perfringens* and is more prevalent type of clostridia genus isolated from the intestinal tract of ostrich (*Struthio camelus*). The pathogenicity of this bacterium is associated with the production of extracellular toxins produced by some of its strains, such as NetB and TpeL toxins. The exact role of these toxins in NE pathogenesis is still controversial. In the present study, *C. perfringens* isolates from healthy and diseased ostrich flocks analyzed to determine the presence of NetB and TpeL toxin genes (*netB* and *tpeL*).

Methods: Thirty six isolates of *C. perfringens* were obtained from NE-positive and NE-negative ostrich flocks in Khorasan-e-Razavi province in Iran and analyzed by two separate single PCR assay to determine the presence of *netB and tpeL* genes. All isolates used in this study genotyping by multiplex PCR and confirmed as type A and C.

Results: Results showed that the *netB* gene was present in 8 (50%) of the 16 isolates obtained from NE outbreaks and were not present in any isolate from healthy ones. The *tpeL* gene was present in 6 necrotic enteritis isolates (37.5%) and 2 healthy ostrich flock isolates (10%).

Conclusion: Statistical analysis using Fisher exact test indicate that there is no statistically significant difference between healthy and diseased flocks for tpeL ($p=0.103$) but for *netB* was ($p<0.001$). These results suggest that *tpeL* is not a necessary virulence factor for the development of necrotic enteritis for many strains of *C. perfringens*. More research is necessary to determine the prevalence of this gene on a global scale, which would indicate its importance as a virulence factor to the *C. perfringens* population as a whole.

IN-SILICO APPROACH TO DESIGN PROTECTIVE VACCINE AGAINST *CLOSTRIDIUM PERFRINGENS*: TARGETING ALPHA AND NETB TOXINS

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Purpose: Necrotic enteritis (NE) is an important infectious disease of poultry caused by gram-positive, anaerobic bacterium *Clostridium perfringens*. Highly immunogenic vaccines including B-cell epitopes of Alpha and NetB toxin might be an option to prevent NE in poultry herds. We evaluated *in silico* approaches using to design recombinant vaccines against *C. perfringens*.

Methods: We retrieved the available Gene-Bank sequences of NetB and Alpha toxins to find signaling peptides and further predicted tertiary structures using homology modeling. Wide array of immunoinformatic servers (in hybrid manner) were used to predict mapping structure of conformational B cell epitopes. The selected consensus including highly immunogenic and reliable B-cell epitope regions then were fused together in a simulatory assay before attaching NetB and Alpha toxin constructs together by linker. We then visualized tertiary structure of recombinant chimeric protein in order to evaluate its efficacy and correct conformation followed by primary structure analysis of construct, post translational modifications, reverse translation, codon optimization, insertion of start/end codon and Kozak sequence and finally open reading frame (ORF) checking.

Results: In total, 3 unique and reliable B-cell antigenic regions were found for each toxin utilizing various wide array of computational analyses and immunoinformatics tools.

The amino acids orders of 98-130, 200-252 and 277-305 for NetB B-cell epitopes and 48-120, 180-215 and 270-373 for Alpha toxin of *C. perfringens* were selected. Finally, the length of designed fusion gene was 1030 bp.

Conclusion: Successful in silico modeling has shown to be promising approach to design robust vaccine targeting Alpha and NetB toxins of *C. perfringens*.

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TOXINOTYPING OF *CLOSTRIDIUM PERFRINGENS* ISOLATES OBTAINED FROM HEALTHY AND DISEASED OSTRICHES (STRUTHIO CAMELUS)

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Purpose: *Clostridium perfringens* is more prevalent type of clostridia genus isolated from the intestinal tract of ostrich (*Struthio camelus*). Necrotic enteritis (NE) is a potentially fatal gastrointestinal (GI) disease of poultry and other avian species, which produces marked destruction of intestinal lining in digestive tract caused by *C. perfringens*. Pathogenicity and lesions are correlated with the toxins produced, thus toxin typing of the bacterium has diagnostic and epidemiological significance. The aim of the present study is to determine which types of *C. perfringens* are among ostrich farms in two categories: diseased and healthy ones and in order to determine the presence of four major toxin genes (*cpa*, *cpb*, *etx*, and *iA*), *cpb2*, and *cpe* genes.

Methods: Thirty isolates of *C. perfringens* were obtained from NE-positive and NE-negative ostrich flocks in Khorasan-e-Razavi province in Iran and analyzed by multiplex PCR assay.

Results: All isolates were positive for alpha toxin gene (*cpa*) and five of those were positive for beta toxin gene (*cpb*). The presence of *cpb2* gene was detected in a high percentage of isolates originating from both healthy (93.3%) and diseased (80%) flocks. No isolate carried enterotoxin gene (*cpe*).

Conclusion: The results suggest that types A and C of *C. perfringens* are the most prevalent types in ostrich in Iran. Due to detection of beta2 toxin gene in isolates from both healthy and diseased birds, it appears that the presence of *cpb2* is not considered a risk by itself.

1250 ***POSTER SESSION II:*** **ANTIMICROBIALS AND RESISTANCE**

Posters will be presented in Poster Session II
Monday, June 30, 1250-1350.

ANTIMICROBIAL ACTIVITY OF SELECTED JORDANIAN MEDICINAL PLANTS

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At the present time, drug resistance in pathogenic bacteria is a major and serious problem. Therefore, plant origin herbal medicines are considered safe and may be effective alternatives to synthetic drugs. In Jordan a large number of plant species that are commonly used by people are reported to have medicinal properties. In this study we focused on three commonly used Jordanian medicinal plants that have been shown to be effective against certain human pathogens and they are: *Peganum harmala L, Pistacia palaestina Boiss,* and *Brassica oleracea L.*

Goals: This study is aimed to determine the antimicrobial activity of three medicinal plants against standard species of *Haemophilus influenza, Helicobacter pylori, Pseudomonas aeruginosa*, *Escherichia coli, Klebseilla pneumonia, Acinetobacter baumannii, Proteus mirabilis, Neisseria meningitides,* and *Staphylococcus aureus* (MRSA).

Methods: Ethanolic extracts of *P. harmala* (aerials), *P. harmala* (seeds), *P. palaestina* Boiss (fruits), and *B. oleracea* (seeds) were tested separately against the growth of the selected bacterial species. Three different concentrations (50 mg/ml, 100 mg/ml and 300mg/ml) were prepared from each extract and the crude extracts were subjected to further fractionation. Extraction of *B. oleracea* (seeds) was achieved using different solvents that included: 95% ethanol, 60 methanol, acetone, ethyl acetate, water and chloroform. Disk diffusion and agar well diffusion methods were used to determine the antimicrobial activity of each extract of different parts of each plant.

Results: The ethanol fraction of *P. harmala* (aerials) showed weak activity against all bacterial strains tested, while the butanol fraction of *P. harmala* (seeds) and *P. palaestina Boiss* (fruits) were the most effective fractions against the tested bacterial strains. While, 60% methanolic extract of *B. oleracea* (seeds) showed the highest activity.

Conclusion: The antibacterial activity of studied plant extracts against the different bacterial species strengthen the scientific evidence of the effectiveness of medicinal plants in the treatment of infections which makes these plants potential candidates for new drugs.

SUSCEPTIBILITIES OF *BACTEROIDES* ISOLATES SUBMITTED TO THE UK ANAEROBE REFERENCE LABORATORY 1999-2013, INITIAL OBSERVATIONS

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Objectives: *Bacteroides* species are the most commonly isolated group of anaerobes from clinical cases. The UK Anaerobe Reference Laboratory receives a number of Metronidazole (MZ) resistant isolates each year, that are also frequently resistant to other common antimicrobials used for treatment of anaerobic infections. This study is a retrospective study of all MZ resistant isolates from 1999-2013 to ascertain the overall level of resistance to the following drugs: clindamycin (CM), co-amoxiclav (Co-A), meropenem (MP) and piperacillin-tazobactam (PTZ). Isolates were also screened for the presence of *nim* genes associated with MZ resistance.

Methods: Susceptibilities were performed on 110 isolates by means of the E test® on Brucella agar. MICs were interpreted using EUCAST/BSAC criteria for gram negative anaerobes (Control strain *Bacteroides fragilis* NCTC 25285 was included with each batch). All isolates were tested genotypically for nim genes using block based PCR and specific primers.

Strains: *Bacteroides fragilis* (n=89), *Bacteroides thetaiotaomicron* (n=11), *Bacteroides ovatus* (n=5), *Bacteroides caccae* (n=2), *Bacteroides xylanisolvens* (n=1), *Bacteroides uniformis* (n=1) *Bacteroides vulgatus* (n=1)

Results: The percentage resistance to each antimicrobial was as follows:
Metronidazole = 100%
Clindamycin = 38%
Meropenem = 24%
Piperacillin-tazobactam = 24%
Co-amoxiclav = 23%
nim genes were detected in 91% of isolates.

Conclusions: In this group of isolates resistance to commonly used antimicrobials was seen in 23 to 38%, with 13% resistant to all 5 antimicrobials (including MZ). These isolates may represent those that could potentially be missed in UK clinical laboratories who screen for 'anaerobes' using an MZ 5 disk, where anything growing up to the zone would be ignored. Subsequently multi drug resistance in this organism may also be missed. The absence of *nim* genes in 9% of these Metronidazole resistant isolates suggests an alternative mechanism of resistance in a minority of these strains.

TRENDS IN THE ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF ANAEROBIC GRAM NEGATIVE BACILLI IN LAGOS, NIGERIA: 1992-2011

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A 20-year data of anaerobic infections in seven prevalent clinical conditions [peritonitis following lower abdominal surgery PLAS (75 cases), periodontal abscess PDA (60), pelvic inflammatory disease PID (22), chronic suppurative otitis media CSOM (21), septic abortion SAB (16), dentoalveolar abscess DVA (12), bloodstream infections BSI (10)] in four specialist hospitals in Lagos, Nigeria and sub-grouped into four periods of five years intervals [1992-1996 PA (21.8% of cases), 1997-2001 PB (28.2%), 2002-2006 PC (29.2%), 2007-2011 PD (20.8%)] were analyzed in reference to antibiotic susceptibility pattern of the prevalent Gram negative bacilli (GNB). The occurrences and distribution of the GNB were *Bacteroides* PA 45, PB 56, PC 67, PD 83, *Fusobacterium* PA 34, PB 46, PC 56, PD 15, *Porphyromonas* PA 3, PB 9, PC 17, PD 1, *Prevotella* PA 36, PB 49, PC 34, PD 8. *Fusobacterium* and *Porphyromonas* were most sensitive to the antibiotics with no evident shift in pattern from 1992 to 2011 but showed highest sensitivity to the cephalosporins and metronidazole. Against *Bacteroides* and *Prevotella*, amoxicillin activity was least with no change in pattern over the study period. Slight but progressive resistance to the cephalosporins by *Bacteroides* and *Prevotella* occurred (22.7% for *B. fragilis* to ceftazidime in 1992-1996 to 28.6 % in 2007-2011). Metronidazole was the most effective antibiotics with resistance not higher than 22.7 % at any time by the most resistant species. The activities of the macrolides increased appreciably from 1992 to 2011 while amoxicillin-clavulanic acid activity was relatively constant. These observations indicate that the changing pattern of antibiotic usage has not appreciably altered the antibiotic profile of anaerobic GNB in Nigeria.

MOLECULAR MARKERS FOR ANTIBIOTIC RESISTANCE IN *BACTEROIDES* AND *PREVOTELLA* TO β-LACTAMS, LINCOSAMIDE AND NITROIMIDAZOLE: A 20 YEAR SURVEY

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Resistance to antibiotics by anaerobic bacteria is on the increase and with observed geographical differences. Use and prescription pattern of antibiotics over time may partly account for resistance within and across geographical boundaries. We compare and contrast the resistance of *Bacteroides* and *Prevotella* to three classes of antibiotics with the presence of resistance genes from 1992-2011. The MIC range and MIC90 of the anaerobes for the antibiotics does not indicate changes in group resistance to antibiotics though isolated cases were recorded. While clear cut patterns were not established for other species, *B. fragilis* resistance increased for amoxicillin-clavulanic acid from 18.2% in 1992-1996 to 31.4% in 2006-2011 and from 27.3% in 1992-1996 to 34.3% in 2006-2011 for cefoxitin. In contrast, decrease susceptibility was obtained against clindamycin (54.5% in 1992-1996 to 22.9% in 2006-2011). Similarly, 22.7% of *B. fragilis* strains have the CepA and/or CfxA gene in 1992-1996 compared to 32.1% in 2006-2011. While not all isolates with CepA and/or CfxA genes were resistant to the β-lactams, all isolates carrying the ermF or nim gene were resistant to the lincosamide (clindamycin) or nitroimidazole (metronidazole) respectively. It is evident that resistance to these groups of antibiotics by *Bacteroides* and *Prevotella* is mostly mediated by these resistance genes.

IMPACT OF TIAMULIN ON *BRACHYSPIRA PILOSICOLI* METABOLISM

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Brachyspira pilosicoli is a common pathogen in poultry farms responsible for avian intestinal spirochetosis (AIS). It usually causes diarrhoea, triggering weight loss and a reduction in egg production and quality. One of the most common treatments used to reduce the impact of *B. pilosicoli* infection is the pleuromutilin antibiotic tiamulin. This antibiotic acts as an inhibitor of protein synthesis. It is widely used in the pig industry and to a lesser extent, in the poultry industry, in order to reduce the spread of intestinal spirochetosis. This study aimed at evaluating the impact of tiamulin on the metabolism of *B. pilosicoli* under optimal growth conditions in order to identify metabolic pathways predominantly triggered by the antibiotic. Bacteria were grown in broth media containing increasing concentrations of tiamulin (from 0.0039 to 0.250 µg/ml) in parallel to a negative control. The experiment was run for five days and we sampled six biological replicates every twenty-four hours for each condition. Purified media were then processed for metabolic profiling analysis by 1H-NMR spectroscopy followed by multivariate statistics. We observed that the bacterial energy consumption and its direct outputs (i.e. butyrate, acetate and amino acids) increased in the media, confirming that glucose fermentation is the major energy source of *B. pilosicoli* in optimal growth conditions. We also demonstrated that the metabolic trajectory followed by *B. pilosicoli* over time was dependent on the antibiotic concentration. For example, the aforementioned glucose consumption by the bacteria was significantly reduced in a dose-dependent manner. In addition, amino acid anabolism (particularly lysine, phenylalanine and valine) was also reduced by the presence of the antibiotic together with the production of short chain fatty acids. These results revealed key bacterial metabolic sensitiveness to the antibiotic stress, which are of interest for future drug development.

A. LAIDLAWII EXTRACELLULAR VESICLES MEDIATE THE EXPORT OF CIPROFLOXACIN AND MUTANT GENE FOR THE ANTIBIOTIC TARGET

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Acholeplasma laidlawii (class Mollicutes) is the ubiquitous mycoplasma, facultative anaerobe being a causative agent for phytomycoplasmoses and the main contaminant of cell cultures. Despite the low efficiency, antibiotic therapy remains the primary tool used for the treatment of mycoplasma infections. The control of mycoplasma infections and elucidation of the rapid development of antibiotic resistance is connected with clarification of mechanisms of mycoplasma adaptation to stress conditions. In our study stress-reactive proteins and genes of *A. laidlawii* PG8 were identified, and it was presented that adaptation of the mycoplasma to environment was connected with the secretion of extracellular membrane vesicles. Recent studies suggest the possible involvement of vesicles in the development of resistance to antibiotics in bacteria, however, similar studies in mycoplasmas have not been conducted yet.

Elucidation of the possibility for participation of extracellular vesicles in the development of resistance to ciprofloxacin in *A. laidlawii* PG8 was the object of the present study.

As a result of our studies it was shown that the level of secretion of vesicles in strain *A. laidlawii* with higher level of resistance to ciprofloxacin was found to be significantly more than that in the original parent strain. The vesicles of this strain contained mutant nucleotide sequences of the target gene of ciprofloxacin (*parC*), exported the antibiotic from cells and displayed bacteriostatic effect toward the ciprofloxacin-sensitive strain of *Staphylococcus aureus*.

Thus our study allows to conclude that development of resistance to ciprofloxacin in mycoplasmas turns out to be related to secretion of extracellular vesicles which mediate export of DNA sequences coding the protein-target for antibiotics and the traffic of ciprofloxacin. The obtained data may facilitate the development of effective approaches to control mycoplasma infections and the contamination of cell cultures.

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ANTIMICROBIAL PROPERTIES OF BASIL ESSENTIAL OIL AGAINST PATHOGENIC BACTERIA

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Currently, there is a strong debate and interest regarding the safety aspects of chemical preservatives added widely in many food products to prevent mainly growth of spoilage and pathogenic microbes. The synthetic compounds are considered responsible for many carcinogenic and teratogenic attributes and residual toxicity. To remedy the aforementioned problems, consumers and the European authorities increased the pressure on food manufacturers to substitute the harmful artificial additives with alternative, more effective, non toxic, natural substances. In this context, the use of natural compounds with antimicrobial activity presents an intriguing case. Hence, the aim of the present study was to investigate potential antimicrobial action of essential oil extracted from basil against foodborne pathogenic bacteria. Basil essential oil was isolated by steam distillation and analyzed by GC/MS. The main components identified were methyl chavicol (*p*-allylanisole) (74.9%) and linalool (18.4%). Subsequently, *in vitro* antibacterial activities were evaluated against *Listeria monocytogenes*, *Salmonella Enteritidis, Salmonella typhimurium, Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The antimicrobial properties were initially assayed by the disk diffusion method and then the minimum inhibitory (MIC) and non-inhibitory concentration (NIC) values were assessed using a microplate reader and an automated technique which combines the absorbance measurements with the common dilution method. Non-linear regression analysis was used to fit the data using a previously published model. The results revealed that basil essential oil is a promising cell growth inhibitor, which may be incorporated in foods as a natural antimicrobial agent.

ANALYSIS OF THE GUT MICROBIOTA OF RATS SUBJECTED TO A TREATMENT WITH VIOLACEIN EXTRACTED FROM *CHROMOBACTERIUM VIOLACEUM*

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The normal microbiota plays a crucial role in the host health by acting as a barrier against invasion of pathogens and contributing with important metabolic functions. Many factors, including diet, antimicrobials and stresses can cause alterations in these populations that, in the intestinal tract, can be associated with inflammatory and allergic disease and metabolic disturbs. *Chromobacterium violaceum* is a Gram-negative bacterium present in the soil and water of tropical and subtropical areas and it produces a pigment called violacein that possess several functions, such as, antibacterial, antiviral, antifungal, and antioxidant activities. Some riverines populations consume these contaminated waters but, nonetheless, they do not seem to develop any type of illness related with this bacterium. Thus, we decide to use an animal model for the initial evaluation of the violacein interference in the gut microbial population. For this experiment, we used three groups of male Wistar rats with 2.5 month. The violacein was extracted from the *C. violaceum* and solubilized in DMSO 5%. This suspension was dilute in sterile water in the concentrations of 50µg/ml (group A) e 500 µg/ml (group B). One hundred microliters of these solutions were administered directly into the mouth of the rats twice a day during 1 month. The control group (Group C) received only water with DMSO 5%. One month later, the rats were sacrificed and the intestinal content was collected for the DNA extraction. A fragment of 23S rRNA was amplified by PCR using universal primers and the amplicons were evaluated by DGGE (50-65%). After the analysis of the gels, we observed that the violacein interfere significatively in the gut microbiota of the rats. Further analysis will be conducted for a better understanding of how the violacein affect this microbiota and whether this change would be beneficial to the host, and may thus come to be used in the treatment of intestinal diseases.

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IN VITRO ACTIVITY OF ANTIMICROBIAL AGENTS AGAINST GRAM-NEGATIVE AND GRAM-POSITIVE ANAEROBIC PATHOGENS COLLECTED FROM THE TIGECYCLINE EUROPEAN SURVEILLANCE TRIAL DURING 2007-2013

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Background: Infections caused by anaerobic bacteria are often mixed, and most frequently occur in the oral cavity and adjacent structures, lower airways, abdominal cavity, female genital tract, and skin and soft tissues. The Tigecycline European Surveillance Trial (TEST) has been monitoring susceptibility of anaerobic pathogens in Europe since 2007.

Methods: 7,405 anaerobic isolates (3,575 Gram-positive and 3,830 Gram-negative) were collected by 102 cumulative sites in 8 European countries from 2007-2013. MIC values for tigecycline and five comparators were determined by a central laboratory using CLSI agar dilution methods. Results were interpreted using EUCAST breakpoints. FDA breakpoints were used for tigecycline as none are defined for anaerobes by EUCAST.

Results: Tigecycline, meropenem, piperacillin-tazobactam, and metronidazole were active drugs against all Gram-positive cocci (*Anaerococcus spp., Finegoldia magna, Parvimonas micra, Peptostreptococcus spp*., and *Peptoniphilus spp*.) with >99.7% susceptibility and MIC_{90} values ranging from 0.12 mg/mL to 0.5 mg/mL. Tigecycline and metronidazole inhibited >90% of *C. difficile* at ≤0.25 µg/mL and 2 µg/mL, respectively. The most predominant Gram-negative species was *B. fragilis* (45.4%). Metronidazole was the most active agent *in vitro* against *B. fragilis* inhibiting >99% at its EUCAST breakpoint of 4 µg/mL. Tigecycline inhibited 97.2% of all *Bacteroides spp*. at its FDA breakpoint of 4 µg/mL. Meropenem, metronidazole, piperacillin-tazobactam, and tigecycline were all active agents against *Prevotella* spp, inhibiting >98% at their respective EUCAST breakpoints.

Conclusions: Tigecycline demonstrated consistent *in vitro* activity against Gram-positive and Gram-negative anaerobes, with the lowest $MIC_{50/90}$ values relative to all comparators over this seven year study period. The EUCAST breakpoint for metronidazole resulted in slightly lower susceptibility rate among *C. difficile* (97.9%) compared to the CLSI breakpoint (99.9%).

LACTOBACILLUS SPP. ISOLATED FROM SHORTNECK-CLAM (*TAPES PHILIPPINARUM*) WITH ANTIMICROBIAL ACTIVITY AGAINST *STREPTOCOCCUS INIAE*

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Streptococcus iniae is one of the major bacterial pathogens of cultured *Paralichthys olivaceus* in Korea. In this study, we attempted to isolate marine lactobacilli strains from Shortneck-clam and analyze their antagonism to *Streptococcus iniae*. Shortneck-clam (*Tapes philippinarum*) samples were collected at the coast of the west sea in South Korea from 2012 to 2013. The shellfish sample was initially plated on Rogosa medium, selective medium for lactobacilli, and the plates were incubated at 37°C for 2 days under anaerobic conditions. Lactic acid production was visually verified based on yellow colonies appeared in lactobacilli MRS (de Man-Rogosa-Sharpe) agar with 0.1% bromocresol purple. A total of 28 lactobacilli strains were isolated and their *in vitro* antagonistic activity against *S. iniae* was investigated using agar diffusion method. Twenty four isolates showed inhibitory activity against *S. iniae*. Four isolates of the 24 were selected based on their high antimicrobial activity against *S. iniae*. Molecular identification of the selected strains is in progress by sequencing of their 16S rRNA gene amplified by PCR. The selected strains will then be further studied for their potential application as aquaculture probiotics.

PII-43

EPIDEMIOLOGICAL ANALYSIS FOR *BACTEROIDES* SPECIES ISOLATED IN JAPAN

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Introduction: Although antimicrobial resistance for *Bacteroides* species has been emergent also in Japan in recent days, there have been a few reports on epidemiological analysis for *Bacteroides* species.

Materials and methods: We have investigated antimicrobial activities for 141 strains of *Bacteroides* species isolated between 2011 and 2012 from 11 medical institutions in Japanese central regions. Bacterial strains were re-identified with matrix-assisted laser desorption ionization-time of flight mass spectrometry. We have examined minimum inhibitory concentrations using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. As for 65 strains isolated in 2012, we have also investigated the presence of carbapenem-resistant coding genes *cepA*, *cfxA* and *cfiA*.

Results: The mean age of the patients isolated *Bacteroides* species was 65.2 years. *Bacteroides* species were isolated from the following samples; 55 strains (39.0%) from pus, 24 (17.0%) from wound and 16 (11.3%) from the blood. There were 100 strains (70.9%) of *Bacteroides fragilis* and 21 strains (14.9%) of *Bacteroides thetaiotaomicron*. The rate of beta-lactamase producing bacteria was 100% for any *Bacteroides* species. Susceptible rates of *B. fragilis* and non-*fragilis* judged by CLSI M100-S22 for SBT/ABPC were 89% and 63.4%, respectively (p=0.0007), for PIPC/TAZ were 100% and 92.7%, respectively (p=0.0233), for CMZ were 71.0% and 7.3%, respectively (p<0.0001) and for CLDM were 56.0% and 17.1%, respectively (p<0.0001). As for carbapenem-resistant coding genes, only non-*fragilis* strains carried *cfxA*. The carriage rate of *cepA* was significantly higher in *B. fragilis* (83.0%) than in the non-*fragilis* strains (0.0%) (p<0.0001).

Discussion: Continuous epidemiological analysis for *Bacteroides* species with resistant coding gene analysis would be necessary in the future.

1245 POSTER SESSION III: VAGINAL MICROBIOME

Posters will be presented in Poster Session III
Tuesday, July 1, 1245-1345.

CHARACTERIZATION OF *CLOSTRIDIUM SORDELLII* AND *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM WOMEN OF REPRODUCTIVE AGE

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C. sordellii and *C. perfringens* can cause fulminant clostridial toxic shock (CTS) following childbirth, abortion or other gynecological procedures. Gynuity Health Projects conducted a study to assess the prevalence and duration of carriage of *C. sordellii* and *C. perfringens* among U.S. reproductive-aged women, and to discover correlates of rectal and vaginal carriage. Women aged 18 – 45 (N=4,939) were enrolled at 25 different U.S. sites. Two vaginal and two rectal swabs were collected from each woman at initial and 2-week visits; those positive at the 2-week visit were sampled again at 6-weeks. Bacterial presence was assessed by *C. sordellii*- and *C. perfringens*-specific PCR and culture. All isolates were submitted to CDC for confirmatory identification, strain typing and characterization. Overall, 4.9% of specimens tested positive for *C. sordellii*, and 11.9% tested positive for *C. perfringens*. Isolates were examined for purity and morphology, and atypical isolates were identified by MALDI-TOF; 238 isolates confirmed as *C. sordellii* and 208 isolates confirmed as *C. perfringens*. Multiplex PCR assays assessed the presence of virulence factors: alpha toxin (CPA), beta toxin (CPB), epsilon toxin (ETX) and perfringolysin (PFO) in *C. perfringens*; phospholipase C (csp), lethal toxin (tcsL) and hemorrhagic toxin (tcsH) in *C. sordellii*. Nearly all *C. perfringens* isolates were CPA-positive (207/208; 99.5%), 172/207 (83.1%) were PFO-positive, and none were CPB or ETX-positive. Among *C. sordellii*, 100% were *csp*-positive, 2 (0.8%) were *tcsL*-positive, and none were *tcsH*-positive. Strain typing demonstrated considerable diversity among both *C. sordellii* and *C. perfringens*, including those cultured from the same patient. *C. sordellii* was isolated more than once from 11 patients, eight carried different strains. *C. perfringens* was isolated more than once from 16 patients, 12 carried different strains. Presence of highly virulent *C. sordellii* and *C. perfringens* is consistent with low rates of infection, but given their seriousness, additional research into the pathogenesis and risk factors for initial colonization and outgrowth may be warranted.

THE ROLE OF THE VAGINAL MICROBIOTA ON THE INFECTIVITY OF SEXUALLY TRANSMITTED INFECTION PATHOGENS

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Background: The composition of vaginal microbial communities affects susceptibility to sexually transmitted infections (STIs). Our recent epidemiologic studies found strong associations between the presence and absence of specific normal vaginal bacterial colonizers and the risk of common STIs. These infections included bacterial vaginosis (BV) (most often caused by *Gardnerella vaginosis*) and *Chlamyida trachomatis* (CT), and *Neisseria gonorrhoeae* (GC). While these associations have been made, it is not well understood how one species of bacteria may affect the ability of another to infect the vaginal mucosa, leading to disease.

The purpose of our work is to understand the mechanisms(s) by which commensal vaginal bacteria inhibit BV/CT/GC infections through the development of an anaerobic *ex vivo* porcine vaginal mucosal infection model.

Methods and Results: Prior vaginal microbiome studies in reproductive aged women identified commensal bacterial strains that may affect the risk of STIs. We are investigating the anaerobic growth and infection dynamics of these clinical vaginal isolates using an *ex vivo* porcine vaginal mucosal (PVM) model (a representative model of the human vaginal mucosa) developed by our laboratory. Our preliminary data demonstrate that commensal *Lactobacillus* strains, *G. vaginalis* and CT grow to bacterial densities on *ex vivo* PVM over 3 days that are similar to densities described intravaginally in women. Furthermore, *Lactobacillus spp.* inhibit the growth of *G. vaginalis* on PVM during a co-culture infection.

Conclusions: This work is important as it describes the growth dynamics of vaginal microbes, *Lactobacillus spp.* and their ability to inhibit the growth of microbes that cause STIs, using a biologically relevant vaginal mucosal surface. We expect our results to have widespread impact on preventative and therapeutic approaches to STIs.

ANTIMICROBIAL ACTIVITY OF BORIC ACID (BA) AND TOL-463 AGAINST VAGINAL ANAEROBES CAUSING BACTERIAL VAGINOSIS (BV) AND URINARY TRACT INFECTIONS (UTIS)

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Objective: TOL-463 is a BA-based vaginal therapy enhanced with EDTA in clinical development for vaginitis. BA is a weak inorganic acid recommended by the CDC in compounded form for recurrent vulvovaginal candidiasis and BV infections. EDTA is a chelator and membrane permeabilizer widely used as an antimicrobial synergist in preservative systems and similarly exploited in the context of TOL463 to potentiate the activity of BA against vaginal pathogens in planktonic and biofilm growth. BA has known activity against vaginal *Candida spp*. but prior to this research, its activity against anaerobic pathogens and lactobacilli was unknown. The present study was thus conducted to characterize the antimicrobial activity of BA alone and with EDTA (TOL-463) on anaerobes implicated in BV and UTIs, and on healthy lactobacilli.

Methods: MICs of BA against anaerobic pathogens and lactobacilli were determined by standard CLSI microdilution methods. 71 genitourinary isolates, including some ATCC strains, were tested. Interaction of BA and EDTA (TOL-463) was then assessed by checkerboard assay and calculation of the fractional inhibitory concentrations index (FICIs). Experiments were run in triplicate or duplicate and included growth and active controls.

Results: BA at 0.4 to 3.2 mg/mL inhibited the majority (~90%) of BV strains, including *G. vaginalis* and *M. curtisii*, which were resistant to metronidazole. Addition of EDTA was synergistic or additive for the majority of *G. vaginalis* and *E. coli* strains and a number of other anaerobic species (FIC 0.26-1.0). The majority of lactobacilli were resistant to BA at the highest concentration, 25 mg/mL. EDTA alone at 0.1 – 0.4 mg/mL inhibited lactobacilli but this effect was antagonized by BA (TOL-463) for the majority of strains tested (FICI 5-9).

Conclusion: TOL-463 is a promising vaginal antiinfective with excellent activity against anaerobes implicated in BV and UTIs, including pathogens resistant to approved treatments, while protecting lactobacilli critical to vaginal health. These results complement earlier work confirming the antifungal activity of TOL-463 against *albicans* and non-*albicans Candida spp*. and its robust antibiofilm effects.

MEDIA FOR PRESERVATION OF MICROBIAL AND IMMUNE BIOMARKERS IN SELF-COLLECTED VAGINAL SWABS

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The deeper understanding of the mucosal immune barrier in the context of vaginal microbiota and human genetic variation compels the need for a reagent that can be simultaneously used to preserve both microbial and immune biomarkers in self-collected vaginal swabs. Under field conditions in community-based studies, the vaginal swabs often need to be collected and stored for variable periods of time at home and transported at ambient temperatures. Therefore, we evaluated the ability of two media commonly used in gene expression and sequencing studies, Thermo Scientific's AssayAssure and Qiagen's AllProtect Tissue Reagent, to preserve DNA, viral RNA and protein levels in vaginal swabs. We specifically assessed vaginal swab recovery of the secretory leukocyte protease inhibitor (SLPI), which has emerged as a marker of the healthy vaginal immunobiome, and chemokines for neutrophils, monocytes, and T cells, e.g. CXCL1 and CCL5, which have been correlated with tissue inflammatory damage and risk of HIV-1 acquisition. Vaginal swabs were submerged in Assay Assure, AllProtect or PBS, and the swabs or their respective elutions were stored at -80°C, 4°C and 37°C for up to 3 days. Total DNA levels, bacterial 16S rRNA, and double-stranded RNA from *Trichomonas vaginalis* virus spiked into test reagents remained stable. The high viscosity of AllProtect was mitigated by a 5-fold dilution in PBS during the swab elution process, which recovered 80% of the total protein from the swabs. The recovery of SLPI, CXCl1 and CCL5 did not decrease significantly over the 3 day period at 37°C. In contrast, in swabs, which were placed in PBS and stored for 24h at 4°C, SLPI, CXCl1 and CCL5 were recovered at 89%, 80% and 62%, respectively, and the protein levels dropped dramatically by more than 80% when stored at 37°C for 3 days. The swabs stored at 37°C in Assay Assure allowed a stable recovery of at least 90% of the chemokine levels and ~70% of the SLPI levels over the 3-day period. These results suggest that both AllProtect and AssayAssure can be used for simultaneous detection of DNA, RNA and proteins stored at ambient temperatures for up to 3 days; however, the recovery may vary by protein over longer periods of time and therefore extended stability studies are needed before reagents are more widely adopted to field studies.

EFFECTS OF VAGINAL LACTOBACILLI ON *TRICHOMONAS VAGINALIS* INFECTION

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Longitudinal studies have shown a significant association between vaginal microbiota lacking significant numbers of lactobacilli and *Trichomonas vaginalis* acquisition. Trichomoniasis, a pervasive sexually transmitted infection, is commonly asymptomatic and is associated with numerous adverse outcomes, including preterm delivery and increased susceptibility to and transmission of HIV infection. The aim of this study was to investigate the *T. vaginalis* interaction with healthy vaginal microbiota analyzing the effects of *Lactobacillu*s strains (*L. brevis* CD2 11988 and *L. salivarius* DSM 24800) on *T. vaginalis* viability and multiplication and the capacity of *T. vaginalis* to phagocytose lactobacilli. In addition, the effect of lactic acid on *T. vaginalis* viability has also been evaluated. Our results demonstrated significant inhibition of *T. vaginalis* multiplication by vaginal lactobacilli. Co-culture assays demonstrated that *L. brevis* inhibited *T. vaginalis* multiplication by 65% and 98.5% after 24 and 48 h incubation, respectively, whereas at the same time points *T. vaginalis* viability was reduced by 20% and 81.7%. *L. salivarius* showed a stronger inhibiting activity: *T. vaginalis* viability and multiplication were inhibited by 90% after 6 h co-culture and by more than 99% after 24 h. Spent culture supernatants obtained from 24 h cultures of *L. brevis* and *L. salivarius,* brought to neutral pH, inhibited *T. vaginalis* multiplication by 78% and 60%, respectively, suggesting that the inhibiting activity exerted by lactobacilli was only partially due to acid production. The amount of viable *L. brevis* and *L. salivarius* were unaffected after 24h co-culture with *T. vaginalis*. Lactic acid inhibited dose- and time-dependently *T. vaginalis* viability and multiplication. Thirty four mM lactic acid determined a 90% inhibition of *T. vaginalis* after 4 h culture, while 17 mM and 4.2 mM caused the same effect after 18 and 24 h, respectively. Our data suggest that a healthy vaginal microbiota can reduce the risk of acquiring *T. vaginalis* infection through the production of lactic acid and other inhibiting molecules produced by lactobacilli.

EFFECTS OF VAGINAL LACTOBACILLI IN *CHLAMYDIA TRACHOMATIS* INFECTION

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Increasing evidence indicates that abnormal vaginal flora lacking lactobacilli facilitates the acquisition of several sexually transmitted diseases including *Chlamydia trachomatis*. *C. trachomatis*, the most common bacterial agent of genital infections worldwide, can progress from the lower to upper reproductive tract and induce severe sequelae. The ability of *C. trachomatis* to develop into a persistent form has been suggested as key pathogenetic mechanism underlying chronic infections and sequelae. The aim of this study was to investigate the *C. trachomatis* interaction with healthy vaginal microbiota analyzing the effects of *Lactobacillu*s strains (*L. brevis* CD2 11988 and *L. salivarius* DSM 24800) on the different phases of *C. trachomatis* developmental cycle. In addition, the effect of lactobacilli on persistent chlamydial forms induced by HSV-2 coinfection has also been evaluated. Our results demonstrated significant inhibition of *C. trachomatis* multiplication by vaginal lactobacilli. *L. brevis* was significantly more effective than *L. salivarius* ($p<0.05$) on all the steps of chlamydial infection cycle suggesting that the ability of lactobacilli to protect from infection is strain-dependent. Lactobacilli had an adverse effect on elementary chlamydial bodies ($p<0.05$), on chlamydial adsorption to epithelial cells ($p<0.001$) and on intracellular phases of chlamydial replication ($p<0.0001$). The study also demonstrated a protective effect of lactobacilli towards persistent *C. trachomatis* forms induced by HSV-2 coinfection. A significant increase in the production of *C. trachomatis* infectious progeny was observed in *C. trachomatis*/HSV-2 coinfection in the presence of *L. brevis* ($p=0.01$) despite a significant inhibition of *C. trachomatis* multiplication ($p=0.028$). Our data suggest that a healthy vaginal microbiota can reduce the risk of acquiring *C. trachomatis* infection and counteract the development of persistent chlamydial forms.

PREDOMINANT *LACTOBACILLUS SPECIES* IDENTIFICATION FROM HEALTHY AND UNHEALTHY FEMALE GENITAL ORGAN BY MOLECULAR TECHNIQUES

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Bacterial infection of female urogenital organ is associated with a range of negative outcomes, including the acquisition of human immunodeficiency virus and other transmitted diseases, preterm births, and pelvic inflammatory disease, in contrast to the Lactobacillus-dominated healthy genital organ. This study was aimed to identify the predominant Lactobacillus species from healthy and unhealthy women. So long, laboratory diagnosis of bacterial infection of women's reproductive organ was dependent on Gram-stained swabs and microscopic observation.

In this study, a molecular method, polymerase chain reaction (PCR) was used for the identification of Lactobacilli species using Lactobacilli species-specific primers: [L.actoF, L.actoR, L.crisF, L.crisR, L.jensF, L.jensR, L.gassF, L.gassR, L.inersF, L.inersR].

Sixty swab samples from healthy women and sixty from infected ranging their ages from 20 – 49 years were donated freshly by Alhabib Hospital and Malaz Clinic, Riyadh, Saudi Arabia during 2012 respectively. Microbial DNA was extracted by two methods, extraction kit and boiling. The PCR products were directly sequenced on an Applied Biosystem 3130 x 1 Genetic Analyzer (Applied Biosystems, Hitachi High-Technologies Corporation Tokyo-Japan). *Lactobacillus crispatus, Lactobacillus gasseri*, and *Lactobacillus iners* were found in healthy and unhealthy women.

The results of this study do not provide support for the findings of YAN Dong-hui *et al.,* in Chinese women and Elahe Motevaseli *et al.,* of Iranian healthy and unhealthy women that *L. crispatus, L. gasseri* and *L. jensenii* were dominant species in healthy women. In contrast to their findings, *L.crispatus, L. gasseri* and *L. iners* were found to be dominant in Saudi healthy women while, *L. jensenii* was dominant only in unhealthy women. In this study, *L. iners, L. gasseri* and *L. crispatus* were found to exist both in healthy and unhealthy women although their dominant numbers were significantly different.

CULTIVATION OF FASTIDIOUS ANAEROBES FROM THE HUMAN VAGINA: DIVERSITY, DYNAMICS & NOVELTY

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Background: Women with bacterial vaginosis (BV) have complex communities of anaerobic bacteria. Several BV-associated bacteria have eluded laboratory propagation.

Methods: Vaginal fluid from women with BV was plated on six different media or inoculated into semi-defined liquid media. Liquid media was supplemented with nutrients such as fatty acids, amino acids, polyamines or human serum. Individual isolates were identified by 16S rRNA gene sequencing. Bacterial community profiles in enrichments were monitored using broad-range PCR and pyrosequencing. Bacterial concentrations during cultivation were measured by quantitative PCR.

Results: We have isolated, identified and maintained >200 distinct strains spanning six phyla. Genomes of ~80 strains are being sequenced by the Human Microbiome Project. Bacteria isolated include: 1) novel strains with <95% 16S rRNA sequence identity to published species (*Dialister* sp. type 2, *Eggerthella*-like), 2) fastidious bacteria of clinical significance to BV (*Prevotella timonensis*, *Megasphaera sp*. type 1), 3) bacteria previously isolated from other body sites but not the vagina (*Jonquetella anthropi*), and 4) phylogenetically diverse isolates of clinical importance (*Prevotella spp*., *Peptoniphilus spp*.). Pyrosequencing showed that different supplements facilitate growth of distinct bacterial species. Moreover, there were changes in bacterial community composition in individual enrichments through the incubation period. Human serum enhanced growth of BV-associated bacterium-3 and *Leptotrichia/Sneathia spp*. by 2-5 log units.

Conclusions: We isolated a diverse set of novel and clinically significant anaerobes from the vagina using approaches that attempt to mirror *in vivo* conditions. Human serum enhanced the growth of several species suggesting that host-derived factors or nutrients are critical for propagation of some bacteria, but other vaginal anaerobes remain uncultivated. PCR methods may be useful for selecting conditions that foster the growth of select fastidious anaerobes.

PCR AND QPCR EXAMINATION OF INTRAUTERIN DEVICES TO IDENTIFY BV-RELATED INDICATOR BACTERIA

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Objectives: The intrauterin contraceptive devices (IUDs) have a signaling thread for removal which are sticking out of the cervix. The normal vaginal bacterial flora is capable of forming a biofilm on it and in time this biofilm is also shown on the IUDs. The pelvic inflammatory disease (PID) has a polymicrobial origin in the vast majority of cases. *Nesseria gonorrhoeae*, *Chlamydia trachomatis* and bacterial vagonosis (BV) pathogens may take a part in the development of it. *Atopobium vaginae* and *Gardnerella vaginalis* are the key pathogens in BV, beside them *Mobiluncus sp.*, *Mycoplasma genitalium* and *Ureaplasma urealyticum* also occur.

Methods: This study was designed to identify bacteria with specific PCR and qPCR methods, which play a role in PID and BV. 75 women were involved who visited the Department of Obstetrics and Gynecology at our University. In the Hungarian Anaerobic Reference Laboratory DNA samples were extracted from the surface of the IUD's using the QIAmp DNA Mini Kit. Extracted DNA was subjected to a human-globin qPCR to monitor for PCR inhibitors and to ensure that amplifiable DNA was present. The bacterial PCR, qPCR assays were carried out on β-globin positive samples. The sequences of the primers used in the study were described earlier in the literature.

Results: 70 samples from 75 can be involved in our study. 51 samples (72.8%) were positive for at least one bacterium. The average numbers of the presented bacterial species were 1 to 4 in the positive samples. In 23 samples (45.1%) one, and in 28 samples (54.9%) two or more species were detected. None of these samples were positive for *N. gonorrhoeae* or *M. genitalium*. *C. trachomatis* was identified only in 1 sample. The number of the positive samples for BV key pathogens were 25 for *A. vaginae*, 43 for *G. vaginalis*, 11 for *Mobiluncus sp.* and 12 for *U. urealyticum*.

Conclusion: We have developed "home made" PCR and qPCR assays for the detection of the key pathogen in PID and BV. We have also demonstrated that the prevalence of *A. vaginae* 49.2%, and of *G. vaginalis* was 84.3% in the examined IUD samples. The utility of the qPCR assay its speed and in the clinical treatment of BV with metronidazole is generally used against anaerobic bacteria, but *A. vaginae* is resistant. This can be an explanation for the failed treatments.

BACTEREMIA CAUSED BY *PREVOTELLA HEPARINOLYTICA* COMPLICATED WITH UTERINE PYOMETRA

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Introduction: *Prevotella* species are obligate gram-negative bacilli that belong to *Bacteroidetes* phylum and have been isolated from the patients with head and neck infections, dental infections, oral infections, respiratory infections, intraabdominal infections and gynecological infections. We have encountered bacteremia by *Prevotella heparinolytica* complicated with uterine pyometra.

Case: A 57-year-old woman admitted to our hospital was receiving steroid therapy for spinal cord sarcoidosis. Around day 43 after admission, the patient complained of irregular vaginal bleeding. She developed left lower quadrant pain, fever up and hypotension on day 74. Since she was clinically diagnosed as peritonitis complicating uterine pyometra. We examined blood and vaginal discharge culture. Drainage for uterine pyometra and antimicrobial chemotherapy with imipenem, clindamycin and teicoplanin, following to clarithromycin have led to complete cure. Blood culture revealed *Prevotella heparinolytica* by 16S rRNA gene sequencing analysis.

Discussion: *P. heparinolytica* has been isolated from the oral cavity in 1969 for the first time, and reported as *Bacteroides heparinolyticus* in 1985. To our knowledge, this is the first report for bacteremia caused by *P. heparinolytica*.

1245 POSTER SESSION III: CLOSTRIDIUM DIFFICILE EPIDEMIOLOGY AND PREVENTION

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CHANGES IN THE INCIDENCE OF *CLOSTRIDIUM DIFFICILE* INFECTION IN A TERTIARY CARE CENTER IN ISRAEL: EMERGING STRAINS OR CHANGES IN DIAGNOSTIC METHODS?

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Objective: To study whether changes in the incidence of *C. difficile* infection (CDI) at Tel-Aviv Sourasky Medical Center (TASMC) resulted from dissemination of the epidemic strain (BI/NAP1/027) or changes in diagnostic methods.

Methods and results: TASMC is a 1200-bed, tertiary center in Tel-Aviv, Israel. The incidence of CDI per 100,000 patient-days declined from 66.9 in 2009 to 43.3 in 2011. Incidence increased to 68.5 in 2012 and declined again to 58.7 in 2013. Throughout this period, CDI cases were kept in contact isolation but were not consistently cohorted. Before 2012, laboratory diagnosis of CDI was based on *C. difficile* toxins (CDT) A&B testing by EIA and criteria for sample acceptance (e.g., rejection of formed stool) were not consistently applied. Diagnostic methods and policies changed in 2012, after national guidelines were issued: initial testing was done by a combined GDH antigen/CDT EIA test followed by CDT PCR in discordant cases, and criteria for sample acceptance were uniformly applied. Consequently, from 2011 to 2012, the proportion of rejected samples increased from 557/3350 (16.6%) to 901/3124 (28.8%) and positivity increased from 211/2793 (7.6%) to 297/2223 (13.4%) ($p<0.001$ for both). The clonal structure was tested on 84 samples that included both community- and hospital-onset CDI cases. Following toxigenic culture, isolates were tested for the presence of the CDT A&B, binary toxin and for the *tcdC* deletion by PCR and were typed by *slpA* sequencing. The epidemic strain (*slpA*-gc8) was found in 5/28 isolates (18%) from 2011 and in 6/31 (19%) from 2013 but was absent in all 25 isolates from 2012. The most common strain (26/84, 31%) was *slpA*-hr (inferred Ribotype 014). The MIC values for both vancomycin and metronidazole were significantly higher in *slpA*-gc8 compared to other strains.

Conclusion: The increase in CDI incidence in 2012 was caused by an improvement in diagnostic methods rather than the dissemination of an epidemic strain.

PREVALENCE OF *CLOSTRIDIUM DIFFICILE* IN THE SUBURBS OF MANGALORE WITH AN EMPHASIS ON EPIDEMIOLOGY AND ACTION OF PHYTOCHEMICALS OF HERBAL ORIGIN

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Purpose: To assess the burden of *C.difficile* by Semi quantitative isolation, characterization, toxin detection with an emphasis on epidemiology and to analyse *in vitro* antimicrobial action of phytochemicals of herbal origin.

Methods & Results: This study was conducted in FMMC Mangalore for a duration of 2 years. The stool samples of 425 patients with diarrhoea were collected from clinically suspected Antibiotic associated diarrhoea, patients with malignancy and paediatric cases. A detailed case history—age, sex, severity of diarrhoea, nature of stool, usage of antibiotic, underlying illness - was taken from the medical records of the respective wards for each patient with informed consent.

Microbiological Analysis: Anaerobic culture on Cycloserine cefoxitin fructose agar (CCFA) and Enzyme immunoassay for the detection of toxins A & B were performed on all the stool samples. Colonies suggestive of *C. difficile* on CCFA were subjected to Latex agglutination and confirmed biochemically. ATCC 43593 *C. difficile* was employed as control strain. PCR is also progressing to know the gene for specific toxin.

Antimicrobial Action of Herbal Extracts: Aqueous, alcohol and chloroform extracts of Aloe vera, tea, coccum and essential oils of clove, ginger, nutmeg were prepared. Antimicrobial action of the extracts against *C. difficile* isolates were tested on Brucella blood agar by disc diffusion & Punch well technique.

Out of 425 samples, 89 (20.9%) were *C. difficile* by culture, 45 (10.6%) were toxigenic which included 15 from 118 (12.71%) paediatric cases 6 from 48 (12.5%) malignancy cases. Ginger, clove, nutmeg and coccum showed good antimicrobial action against the isolates.

Conclusion: The prevalence of *C. difficile* is either overlooked or under reported from India. As the colonizer strains can also be toxigenic, caution should be taken while reporting. Natural herbal products without side effects may be promising in the treatment, however requires more studies to prove the same.

DETECTION OF *CLOSTRIDIUM DIFFICILE* AND MICROBIOTA COMPOSITION IN ELDERLY HOME RESIDENTS FROM SLOVENIA

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Clostridium difficile infection (CDI) is found in all age groups but still mostly associated with patients older than 65 years, indicating that elderly homes could be an important reservoir.

From three elderly homes in Slovenia 88 residents (from 60 to 98 years) were enrolled in the study. *C. difficile* was detected either by specific 16S rDNA RT PCR or by culturing. Out of 88 faecal samples, 6 (6.8%) were positive for *C. difficile*. The presence of positive samples in individual elderly homes was 5/30 (16.7%), 0/29 (0%) and 1/27 (3.7%). By PCR ribotyping 3 different PCR ribotypes were determined, 011/049, 010 (nontoxinogenic) and SLO 188. Two samples were positive by 16S rDNA RT PCR, but were negative on direct PCR ribotyping.

In a subset of samples human gut microbiota was also characterized by a denaturing high performance liquid chromatography (DHPLC) and/or pyrosequencing. Up to 180 (DHPLC method) and up to 470 (pyrosequencing method) different bacterial species were identified.

Differences in gut microbiota of *C. difficile* positive and negative individuals included decreased relative abundance of *Firmicutes*, *Verrucomicrobia* and *Bacteroidetes* in *C. difficile* positive individuals and increase in relative abundance of the *Proteobacteria*. According to DHPLC method also the presence of the phylum *Actinobacteria* slightly increased in the *C. difficile* positive individuals, whereas results from pyrosequencing indicated no significant differences between *C. difficile* positive and *C. difficile* negative individuals.

CLINICAL CHARACTERISTICS OF *CLOSTRIDIUM DIFFICILE* INFECTION IN HOSPITALIZED PATIENTS WITH ANTIBIOTIC-ASSOCIATED DIARRHOEA IN A UNIVERSITY HOSPITAL IN CHINA

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Purpose: To identify clinical characteristics of *Clostridium difficile* infection (CDI) in patients with antibiotic-associated diarrhea (AAD).

Methods: A prospective study was conducted among patients hospitalized in Fudan University Hospital Huashan from August 1, 2012 to July 31, 2013. Toxigenic *C. difficile* isolates were characterized by PCR ribotyping and multilocus sequence typing.

Results: AAD developed in 1.0 % (206/20437) of the antibiotic-treated patients and a toxigenic *C. difficile* isolate was recovered from 30.6% (63/206) of diarrhoeal stool samples. The frequency of AAD was highest in the ICU (10.7%), however the proportion of CDI in AAD was highest in the Geriatric Unit (38%). All diarrhoea was hospital-acquired and ranged in severity from mild to moderate; one case with pseudomembranous colitis was identified. Use of carbapenems (OR, 2.31; 95% CI, 1.22 to 4.38; P= 0.011) was found to significantly increase the risk of CDI; patient demographics, presumed risk factors, clinical manifestations and laboratory findings revealed no significant difference between patients with CDI and non-*C. difficile* AAD. More than 90% of patients with CDI or non-*C. difficile* AAD were cured; only 2 patients had CDI recurrence. Ribotype H was the dominant (18.8%) genotype, followed by ribotype 012 and ribotype 017.

Conclusions: *C. difficile* plays a significant role in AAD in our setting in China. Because the severity of diarrhoea ranges from mild to moderate it is difficult for Chinese clinicians to identify CDI from AAD patients, therefore CDI should be included in the routine differential diagnoses for hospitalized patients presenting with AAD.

LONGITUDINAL AUTOPSY STUDY (1975-2010) OF *CLOSTRIDIUM DIFFICILE* INFECTION AND PSEUDO-MEMBRANOUS COLITIS

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Clostridium difficile (CD) turned out to be an etiology of human pseudomembranous colitis (PMC) in late-1970s. We could successfully isolate CD from PMC patient in May, 1979, and since then, we launched surveillance of CD infection in a geriatric acute-care hospital in metropolitan area. Throughout 36 years (1975-2010) of surveillance, we elucidate its epidemiology and implications in mortalities in our autopsy cases with CD infection and PMC.

Methods: TMGH has continuously conducted the best efforts to maintain the quality and quantity of autopsy and pathologists have maintained the standard criteria for judging PMC. We reviewed the autopsy reports of PMC patients and analyzed underlying diseases, clinical course, and cause of death combined with CD culture and/or CD toxin assays.

Results: Overall autopsy rate was about 80% in 1970s, and then it came to about 20% in 2010. In the period of 1975-1983, 2000-2003, and 2006-2010, PMC was observed in 1.6% (32/2,000), 4.0% (27/670), and 2.8% (14/498) of all autopsy cases, respectively. CD infection or PMC was not diagnosed prior to death in 33.3% (9/27) and 28.5% (4/14) in 2000-2003 and 2006-2010, whereas PMC was the direct and independent cause of death in 25.9% (7/27) and 14.2% (2/14) of autopsy cases, respectively. Even in 2006-2010, 21.4% (3/14) CD infection was diagnosed within one week before death.

Conclusion: Although BI/NAP1/027 strain has been sporadically reported in Japan so far (ribotype smz/018 is mainly endemic), and diagnostic techniques advanced during these 36 years, PMC continue to attribute to substantial mortalities in geriatric populations. Vancomycin therapy had limitations including adherence to oral therapy, impaired intestinal motilities, and end-stage underlying conditions. Comprehensive and continuous efforts for reducing CD infection are still needed in elderly patients.

MOLECULAR BIODIVERSITY OF *C. DIFFICILE* ISOLATES IDENTIFIED FROM HOSPITAL ROOMS WITH OR WITHOUT DIAGNOSED *C. DIFFICILE* ASSOCIATED DIARRHEA (CDAD)

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CDAD is a leading cause of antibiotic-associated diarrhea and is endemic in hospitals without clear environmental source or routes of transmission. *C. difficile* (CD) spores persist in hospital environments for an extended period showing uncontrolled transmission despite costly prevention strategies. We determined the presence of environmental CD adjacent to patients with symptomatic CDAD. This study aimed to investigate ward-based transmission of *CD*, by subdividing organisms into distinct lineages defined by ribotyping. It was a prospective cohort study, carried out in a large teaching hospital in Houston, TX. When CDAD cases were identified, the affected patients' rooms were cultured for CD using environmental sponge followed by conventional laboratory isolation methods which was repeated weekly for 3 weeks after the infection was first diagnosed. Environmental sample collection was stopped if a negative environmental culture was obtained. CDAD was defined as passage of at least three unformed stools per day together with *C. difficile* culture- or toxin-positive stools. Environmental samples were obtained from surfaces adjacent to the patient and from communal areas of the room. *C. difficile* isolates were characterized by ribotyping to determine relatedness. Over a 3-month period (June – August 2012), we conducted environmental sampling in rooms of 55 patients with CDAD and in 55 control rooms immediately adjacent to the rooms of patients with CDAD. Of the 55 case rooms, 25 (45%) were positive for CD. For the 55 control rooms 6 (11%) were positive for environmental CD. All CD isolates (25 from CDAD patients' room and 6 from control room) were ribotyped. A similarity index of ≥75% was used to define clusters when comparing the known 7 CD reference strains determined by PCR ribotyping. Nine different PCR-ribotype clusters were identified. No 027 ribotype was identified from the environmental sampling. Environmental sampling collected during the follow-up period, 3 of the 6 rooms showed same ribotyping cluster for at least 2 weekly samples. *C. difficile* occurred commonly in the hospital environment housing patients with and without CDAD. The prevalent strains showed numerous ribotype patterns suggesting occurrence in a hospital of many small clusters of CD strains rather than presence of one or more strains with wide transmission. The findings of this study have implications for our understanding of the epidemiology, transmission, and environmental control of hospital CDAD.

CHARACTERISATION OF *CLOSTRIDIUM DIFFICILE* STRAINS ISOLATED FROM PATIENTS ATTENDING THE GROOTE SCHUUR HOSPITAL, CAPE TOWN, SOUTH AFRICA

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In South Africa there is no routine surveillance of *Clostridium difficile* and little is known regarding its prevalence in the local hospital environment. Therefore, in order to obtain epidemiological information for South Africa, *C. difficile* strains were selectively isolated from patients with diarrhoea (n = 166) attending the Gastroenterology Clinic at Groote Schuur Hospital. Isolates were typed using a combination of ribotyping and multilocus variable-number tandem-repeat analyses (MLVA) and their susceptibilities to antibiotics (metronidazole, vancomycin, erythromycin and moxifloxacin) determined by Etest strips. Screening for previously identified antibiotic resistance genetic determinants was also carried out on resistant isolates. Toxigenic *C. difficile* was isolated from 34/166 samples giving an overall carriage rate of approximately 20%. Isolated strains predominantly belonged to the ribotype 017 group, many of which showed resistance to multiple antibiotics. MLVA data suggested unique sources of infection as well as patient to patient transfer. Resistance to erythromycin and moxifloxacin was correlated with the presence of the *ermB* gene and mutations in the *gyrA* gene, respectively. Strains belonging to the ribotype 017 group showed very strong auto aggregation in *in vitro* broth cultures, which may play a role in enhancing gut colonisation by the bacterium. These results reflect the diversity of strains present at a single hospital and provide a basis for further analyses across a greater number of sample sites in South Africa.

POPULATION-BASED COHORT STUDY OF *CLOSTRIDIUM DIFFICILE* INFECTION (CDI) IN THE UNITED STATES

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Statement of Purpose: To determine the population-specific CDI burden in the US Medicare population.

Methods: Retrospective cohort study of CDI in the Medicare 5% random sample in 2009. Comorbidities and infections (including CDI) were determined based on International Classification of Diseases, Ninth Revision, Clinical Modification diagnosis codes. A patient's first episode of CDI in 2009 was identified. The incidence per 100,000 population and population attributable risk percent (PAR%) of CDI were determined for non-CDI infections, healthcare exposures, and comorbidities.

Results: There were 1,465,927 people, 8,992 had >=1 episode of CDI (613/100,000 persons). In general, the CDI incidence was highest among patients with a non-CDI infection in the previous three months. The three infections with highest CDI incidence per 100,000 persons were septicemia (19,511), non-CDI gastroenteritis (12,864), and surgical site infection (11,332). The infections with the three highest PAR% were urinary tract infection (40.8%), pneumonia (36.1%), and septicemia (25.7%). The three healthcare exposures with the highest subsequent CDI incidence were an emergency hospitalisation in the previous three months (7,526), dialysis in the prior three months (5,025), and stay in a nursing home in the prior six months (4,832). The three healthcare exposures with the highest PAR% were an emergency department visit without an admission in the last year (73.2%), an emergency hospitalization in the last year (73.0%), and at least one hospitalisation in the last year (70.9%). The three comorbidities with the highest CDI incidence were weight loss (4,541), fluid and electrolyte disorder (3,541) and blood loss anaemia (3,198). The three comorbidities with the highest PAR% were hypertension (60.5%), fluid and electrolyte disorders (40.5%), and deficiency anaemia (37.4%).

Conclusion: Population specific incidence and PAR% for CDI were identified. The highest incidence and PAR% conditions were not always the same, as PAR% takes into account prevalence of the condition. These data can be used to target population-based CDI prevention efforts.

PREDICTING FACTORS FOR DEVELOPMENT OF *CLOSTRIDIUM DIFFICILE* INFECTIONS

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A Statement of the Purpose: In recent years, *Clostridium difficile* has become the main cause of bacterial infectious diarrhea in nosocomial settings.

Methods: A retrospective study was performed on 252 patients with *Clostridium difficile* infections (CDI) in General Hospital Uzice, between 01.01. 2009 and 31.12. 2013. We reviewed the following aspects regarding *Clostridium difficile* infections: epidemiology, clinical course, treatment. Diagnosis was established by confirmation of *Clostridium difficile* toxins A and B using immunochromatographic rapid test.

Results: The incidence rate of CDI was 0.1% in 2009, 0.2% in 2010, 0.3% in 2011, 0.9% in 2012, and 1.1% in 2013. Mean age at onset was 75.4+/-8.1 (range: 30-89), and 232 (92.1%) patients were older then 65yr. Before onset of CDI, 222 (88.1%) patients were treated by antibiotics: 39% were treated by ciprofloxacin, 35% by clindamycin, and 26% by cephtriaxon. Median duration of antibiotic treatment was 12 days. Chronic illness, like diabetes mellitus, chronic cardiovascular, pulmonary, hematological, or liver diseases and alcoholism, were notified in 197 (78.2%) patients. The complications of the disease were notified in 122 (48.4%) patients with CDI: kidney failure 110 (90.2%) and toxic megacolon 11 (9.0%). The case fatality was 1 (0.8%). Age, antibiotic treatment and chronic illness are evaluated as predicting factors for development of CDI. Multivariate linear regression analysis revealed age older 65 as the most important positive variable for development of CDI (B 0.408; S.E.0.104; P<0.01).

Conclusion: Patients with CDI are older aged, with chronic illness, and significantly more frequently treated by antibiotics. Older age is the principal predicting factor for the development of CDI in hospitalized patients.

AN EMERGING *CLOSTRIDIUM DIFFICILE* STRAIN WITH INCREASED VIRULENCE NOT ASSOCIATED WITH TOXIN HYPERPRODUCTION

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The prevalence of *Clostridium difficile* infections has increased due to the rise of hypervirulent strains. An emerging strain from a novel NAP type was isolated from an outbreak in a Costa Rican hospital and denominated NAP_{CR1}. This strain was responsible for one third of the cases in this outbreak and induced a severe clinical presentation. The increased virulence of NAP_{CR1} was confirmed in the hamster model where it behaved as the hypervirulent NAP1 strain. As NAP1, NAP_{CR1} strain is resistant to fluoroquinolones and possesses an 18 bp deletion in *tcdC*. On the other hand it does not harbor the binary toxin and does not produce increased levels of toxin A and toxin B, by western-blot and qRT-PCR. Bacterial-free supernatants from NAP_{CR1} strains induced strong inflammatory responses in the murine ileal loop model and high mortality rate in hamster's infection model indicating that molecular determinants produced and secreted by this particular strain could synergize the action of the toxins thus explaining its increased virulence.

SURVEILLANCE OF ANTIBIOTIC RESISTANCE TREND AMONG HOSPITAL AND COMMUNITY-ACQUIRED TOXIGENIC *CLOSTRIDIUM DIFFICILE* ISOLATES OVER A 5-YEAR PERIOD IN KUWAIT

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Introduction: *Clostridium difficile* is a leading and important cause of diarrhea in industrialized countries.

Objective: To investigate the trend of antibiotic susceptibility of hospital-acquired and community-acquired *C. difficile* (HA-CD; CA-CD) over 5 years (2008-2013).

Methods: Stool samples were collected from diarrheagenic patients suspected of *C. difficile* infection acquired in the hospital or community and processed in the Anaerobe Reference Laboratory (ARL), Faculty of Medicine, Kuwait. Stools were cultured on selective media after heat-shock procedure. Suspected *C. difficile* isolate were identified by Gram staining, biochemical tests using API 20A and confirmed by VITEK MS. Toxin production was investigated by GeneXpert™ and EIA methods. Antimicrobial susceptibility testing (AST) was performed by determining the minimum inhibitory concentrations (MICs) of a variety of antibiotics using the E test method according to the manufacturer's instructions. Resistance profiles of the isolates were determined according to the interpretative criteria recommended by the CLSI (2014).

Results: A total of 111 hospital acquired and 35 community-acquired *C. difficile* isolates were analyzed. Amoxicillin-clavulanic acid, daptomycin, linezolid, metronidazole, piperacillin- tazobactam, teicoplanin, tigecycline and vancomycin all had excellent activities against the HA-CD and CA-CD isolates. Penicillin resistance was more among the community isolates compared to the hospital isolates (100 vs. 58.9%), respectively. The HA-CD isolates were more resistant to clindamycin (52.6%) than the CA-CD (43%) with a decreasing trend over years. Overall resistance of HA-CD and CA-CD isolates to imipenem and meropenem were 47.3 and 0% and 100 and 43%, respectively. In general, while the resistance rates of HA-CD isolates to most antibiotics were decreasing over time, those of CA-CD were on the ascendancy.

Conclusions: We did not encounter any vancomycin or metronidazole resistance amongst the HA-CD and CA-CD isolates during this series. While resistance rates to most of the anti-anaerobic agents have decreased over years among the HA-CD isolates, there has been increasing trend among the CA-CD. We recommend periodic surveillance and regular AST for all toxigenic *C. difficile* isolates as an informed guide to empiric antibiotic use.

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INCREASED MULTI-DRUG RESISTANT *CLOSTRIDIUM DIFFICILE* IS DRIVEN BY THE PREVALENCE OF ARL 027 AND ITS DOMINANCE IN THE NURSING HOMES

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Approximately 7900 consecutive fecal samples from inpatient (IP), outpatient (OP), and nursing home patients (NH) were submitted to a southwestern Virginia hospital laboratory and anonymous, unlinked, excess material from each was screened for the presence of *C. difficile*. 2072 isolates were recovered and PCR ribotyped. The minimum inhibitory concentration (MIC) of metronidazole (MZ), vancomycin (VA), rifampicin (RIF), moxifloxacin (MOX), clindamycin (CL), and erthyromycin (ERM) were measured using the Etest. Ribotypes and antibiotic susceptibilities were analyzed by prevalence and by patient populations. ARL 027, 014, and 053 comprised the top three ribotypes, respectively. MIC_{50}, MIC_{90}, and geometric mean were calculated and multi-drug resistance (MDR) phenotypes were identified, ranging from sensitive isolates (MDR-0) to strains resistant to 4 drugs (MDR-4). NH had the highest rate of *C. difficile* (48.5%), while IP and OP were in the 20-25% range, a standard expectation of *C. difficile* prevalence. OP had the highest Shannon-Weiner diversity index (SWDI) of 3.338, the lowest prevalence of 027 (18%), the highest prevalence of 014 (13%), a very low prevalence of 053 (2%), and was dominated by MDR-0 isolates (63%). NH had the lowest SWDI (2.086), the highest prevalence of 027 (53%), the lowest prevalence of 014 (8%), and was mostly phenotype MDR-4 (38%). IP resembled an average of the OP and NH. Its SWDI was 2.853, the prevalence of 027, 014, and 053 was 33%, 10%, and 11%, respectively, and IP had a larger mix of MDR types, although it was mostly MDR-0 (41%). The MIC_{50} and MIC_{90} of NH and ARL 027 were greater than or equivalent to the other groups for each of the antibiotics tested. NH had a significantly higher MIC geometric mean versus IP and OP for MOX, RIF, ERM, and CL (all were p=≤.05) and also versus OP for VA (p=.0058) and for MZ (p=.0153). ARL 027 had a significantly higher MIC geometric mean versus 014 and 053 for MOX, VA, RIF, ERM, and for CL (all were p=≤.05) and also versus 014 for MZ (p=≤.001). Increased multi-drug resistance is driven by the high prevalence of 027 which in turn is influenced by its dominance within the NH community.

1245 ***POSTER SESSION III: CLOSTRIDIUM DIFFICILE TREATMENT AND IMMULITY***

Posters will be presented in Poster Session III
Tuesday, July 1, 1245-1345.

RESOLUTION OF RECURRENT *CLOSTRIDIUM DIFFICILE* INFECTION (RCDI) USING A STAGGERED ANTIBIOTIC WITHDRAWAL PROTOCOL AND KEFIR

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The incidence of RCDI has increased dramatically in the last decade. Although fecal microbiota therapy (FMT) is highly effective, many patients are unable to afford the cost or are adverse to the esthetics of FMT and alternative treatment strategies are needed.

Materials and Methods: Retrospective study of 26 patients (22 females, 4 males, mean age 67 years) with RCDI who had reestablished normal bowel function following standard treatment with oral metronidazole or vancomycin and Lifeway Kefir®. All patients continued treatment with a tapered antibiotic withdrawal regimen while continuing to ingest Kefir (a probiotic) with meals for an additional 6 weeks. The antibiotic dose was gradually reduced every 2 weeks, and each antibiotic dose was taken at 72 hour intervals.

Results: 22 patients (84%) permanently resolved their recurrent diarrhea and had not experienced a recurrence by 1 year after completion of treatment. The remaining 4 patients relapsed within 45 days of treatment, but permanently resolved their infection after a standard 2 week course of vancomycin immediately followed by a 2 week course of rifaximin.

Conclusion: Patients with RCDI who are unable to afford the cost or who elect to not be treated with FMT may successfully resolve their relapsing infection with a staggered antibiotic withdrawal treatment regimen in combination with daily ingestion of Kefir.

LOW CARRIAGE RATES OF *CLOSTRIDIUM DIFFICILE* AMONG HERBS USERS IN LAGOS, NIGERIA

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The past decades have witnessed a tremendous increased incidence and severity of *Clostridium difficile* infection (CDI) especially in the Western countries. Such incidence is almost not known or nonexistence in Nigeria where there is a wide use and misuse of antibiotics suggesting that CDI could be widespread in the country. A study was therefore carried out to determine the carriage rates of *Clostridium difficile* between hospital and community patients in four randomly selected Local Governments Area (LGA) of Lagos which included Agege, Ikeja, Mushin and Surulere. A total of 763 stool samples made up of 152 from Agege, 223 from Ikeja, 197 from Surulere and 191 from Mushin local government were collected from both children and adults. The result showed a high colonization rate of *C. difficile* among the different age groups and an overall prevalence of 20.4% (156 of 763) of *C. difficile* and its toxin isolated. The highest carriage rate was seen among patients from hospitals in Ikeja and in individuals in the community in Surulere LGAs though the difference was not statistically significant ($p < 0.491$). There appeared to be an inverse relationship between the use of local herbs and *C. difficile* acquisition as more than 60% ($p< 0.001$) of the study population who were on local medication were not colonised. From this study, it appears *C. difficile* does not pose any threat among Nigerian populace compared to that seen in the Western countries despite the high indiscriminate use of antibiotics. This could be as a result of the fact that most laboratories do not have facilities for the detection of this organism as such do not screen for it but for culture of routine enteropathogens more so, the high usage of medicinal plants among the local populace especially the elderly may have contributed to low incidence of CDI in this region. However, just like other diseases like cancer that previously was not regarded as a health problem due to lack of diagnosis, but as a result of advances in medical diagnosis and management have become common and frequently diagnosed in our society, *C. difficile* may soon be recognised as an important pathogen in clinical practice if more awareness is created and its testing becomes regular in the health sector. It is therefore important that periodical surveillance be constituted to monitor this organism.

CLOSTRIDIUM DIFFICILE: DIAGNOSTIC AND THERAPEUTIC PROBLEMS

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Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacilli and is now the leading cause of health-care-associated infections, surpassing MRSA. In recent years, a new focus has been put on *C. difficile* due to the emergence of hyperepidemic strains that have led to outbreaks around the world and an increasing incidence of disease. Risk for the development of *C. difficile* infections—CDI is mainly associated with the use of broad spectrum antibiotics as well as increasing patient age and hospitalization.

The aim of this study was to analize different methods and diagnostics algorythms for CDI. 91 stool samples obtained from 55 woman and 36 men (median age 68.6 years) with diarrhea of unkown etiology were studied. Diagnostic tests: Quik chek complete, Tox A/B TechLab (USA), and Illumigene *C. difficile* loop-mediated assay (Meridian, USA) were used for diagnostic purposes according to manufacturer's instruction. Culture of *C. difficile* was performed on CCCA agar (bioMerieux, France). Fecal lactoferrin level was determined by TechLab Elisa test. Results were analyzed using Statistica 2009 program.

Twenty eight (30.8%) out of 91 studied stool samples demonstrated negative results in all used assays, and 12 (13.2%) demonstrated positive results in all used methods. In 27 cases (29.7%) only antygen GDH was positive, in 1 (1.1%) sample Quik chek complete (GDH and Tox B) was positive. In 5 (5.5%) samples GDH, Tox A/B and Illumigene *C. difficile* were positive. In 1 case (1,1%) only Illumigene *C. difficile* was positive. In 17 samples (18.7%) GDH and Illumigene *C. difficile* assays were positive.

Presence of *C. difficile* toxins in fecal samples, demonstrated by TechLab Tox A/B test strongly correlated with clinical symptoms of antibiotic-associated diarrhoea, as well as with elevated fecal lactoferrin level.

IN VITRO ACTIVITY OF VANCOMYCIN (VA), METRONIDAZOLE (ME), CLINDAMYCIN (CL), MOXIFLOXACIN (MO), FIDAXOMICIN (FI) AND RIFAXIMIN (RX) AGAINST *CLOSTRIDIUM DIFFICILE* ISOLATES RECOVERED FROM PATIENTS ENROLLED IN A CLINICAL TRIAL OF HUMAN MONOCLONAL ANTIBODIES TO TOXINS A AND B

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Background: *Clostridium difficile* continues to be a major nosocomial pathogen and has spread into the community where increasing numbers of infections are being reported. New approaches to treatment are being developed.

Methods: The R.M. Alden Lab received 1030 pre-treatment fecal samples from patients enrolled in a Phase III clinical trial of Efficacy, Safety and Tolerability of Human Monoclonal Antibodies to *C. difficile* Toxins A and B. Study sites included 27 countries in North and South America, Europe, Israel, and Asia. Antibiotic treatment with VA, ME, or FI was at the discretion of the investigator. Fecal samples were collected at the time of enrollment, tested for toxin at the study site, frozen, and sent to a central lab for subsequent shipping to our lab for further testing. We cultured the samples, performed susceptibility tests with six antimicrobial agents using the agar dilution method, and shipped copies of isolates to two additional labs for REA and ribotyping.

Results: Decreased susceptibility to VA (MIC 4-8 µg/ml) was seen in 101/1030 isolates (9.8%). These were predominantly from USA and Israel, with ribotypes most commonly 027 and 137, respectively. Resistance to MO was seen in 91 of these 101 isolates (90.1%), and resistance to CL was present in 95 (94.1%). Decreased susceptibility to ME (MIC 4-8 µg/ml) was found in 27 of 1030 strains (2.6%), which also had total resistance to MO (100%) and increased resistance to CL (74%). These strains were mostly ribotypes 027 and 001/072 that were recovered largely from sites in Chile, Russia, Poland, and USA. RX resistance (MIC >32µg/ml) was seen in 12.6% of isolates overall; just over a third of these (44/130) were from USA sites with ribotype 027 predominating. Italy, Spain and Poland were well represented with ribotype 001/072, and REA Groups J and W. One strain with FI MIC of >8 µg/ml was recovered from a site in the USA. **Conclusions:** Decreasing susceptibility to commonly used antimicrobials for treating *C. difficile* infection was present in clinical isolates recovered from patients enrolled in this study. Fecal concentrations of standard of care antibiotics are likely to exceed the MICs. Nevertheless, new approaches are needed to address the challenges to successful treatment of this important infection.

INVESTIGATION OF THE MICS OF FIDAXOMICIN AGAINST HUNGARIAN *CLOSTRIDIUM DIFFICILE* CLINICAL ISOLATES

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Clostridium difficile infection (CDI) usually occurs after exposure to broad-spectrum antibiotics and is the most common identifiable cause of diarrhea in hospitalized patients. Current treatment strategies are inadequate with decreased response rates to metronidazole, and high recurrence rates with the use of metronidazole and oral vancomycin. Fidaxomicin is a new macrocyclic antibiotic, which has a narrow spectrum of activity against gram-positive anaerobes and is bactericidal against *C. difficile*. Fidaxomicin stands out as the first-in-class oral macrocyclic antibiotic with targeted activity against *C. difficile* and minimal damage on the normal colonic flora.

The aim of this study was to investigate the *in vitro* activities of fidaxomicin against 188 *C. difficile* strains isolated in our laboratory or in different centers of Hungary. The strains' toxicity was tested routinely. The determination of MICs of metronidazole, moxifloxacin, rifampicin and vancomycin has already been done previously by E-test method. MICs of fidaxomicin were determined by agar dilution (according to the CLSI recommendations). None of the isolates were resistant for metronidazole or vancomycin. However, 35 of the 188 isolates (18.6%) proved to have an MIC>32 µg/ml for moxifloxacin and 19 (10.1%) had an MIC≥32 µg/ml for rifampicin. The *C. difficile* isolates displayed minimum inhibitory concentrations (MIC) for fidaxomicin in the range of <0.008-0.5 µg/ml, with a MIC_{90} of 0.125 µg/ml. Only four isolates (2.1%) had 0.5 µg/ml MICs to fidaxomicin. The detected MICs displayed an identical distribution with respect to the EUCAST database for wild-type strains. The MICs of fidaxomicin for the control *C. difficile* strain (*C. difficile* 630) was 0.064 ± 1 dilution.

Overall, fidaxomicin proved to be a highly effective drug against *C. difficile in vitro* outscoring the efficiency of metronidazole and vancomycin.

IMMUNOTHERAPY FOR SEVERE *C. DIFFICILE* INFECTION

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Over the past decade, *Clostridium difficile* has become the most common cause of nosocomial disease in the healthcare environment. Epidemic strains have emerged which exhibit enhanced virulence factors including resistance to fluoroquinolones, an increased capacity to form highly resistant spores and produce significantly more toxin A (TcdA) and toxin B (TcdB) than conventional strains. These factors help explain why *Clostridium difficile* infection (CDI) is becoming more frequent, severe and difficult to treat.

Recently, formaldehyde treated recombinant fragments, based on TcdA (TxA4) and TcdB (TxB4), have been used to raise polyclonal antibodies (PcAb) in sheep. These PcAb were shown to have high toxin neutralising titres against their respective native toxins in a cell based assay, and provided protection against CDI in the hamster model. In this study, we assessed a range of doses of either TxA4 or TxB4 to immunise 2 flocks of 15 sheep and monitored the optimum dose required to elicit high titres of toxin neutralising PcAb. Both flocks were sub-divided into five groups, each receiving a different dose (from 50µg to 2mg), with serum samples taken and re-immunisation given at four week intervals. PcAb binding titre was assessed by enzyme immunoassay (EIA) and potency was measured by a cell based cytotoxic neutralisation assay.

TcdB was found to be more cytotoxic than TcdA with the effective concentration required to kill 50% of the cells (LC_{50}) measured at 1000 pg/mL compared to 16 pg/mL for TcdA and TcdB respectively. In the dose response study, TxA4 appears to be a better immunogen with neutralising antibody titres of ~ 100,000 compared to ~ 40,000 for TxB4.

Binding titre was found to be higher than neutralising potency in all dose groups in the first 14 weeks of immunisations with no dose related response evident. However, at week 22 the neutralising potency of the higher dose groups began to trend upwards.

These results demonstrate that toxin neutralising PcAb can be raised in sheep and that higher immunising doses result in increased antibody response. This is a step towards the development of an immunotherapeutic for the treatment of severe cases of CDI.

PIII-29

SUSCEPTIBILITY OF *CLOSTRIDIUM DIFFICILE* CLINICAL ISOLATES TO METRONIDAZOLE, VANCOMYCIN AND CLINDAMYCIN IN TERTIARY HOSPITALS IN MEDELLÍN–COLOMBIA

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Clostridium difficile is the causal agent of a broad spectrum of intestinal diseases in the hospital environment, producing high rates of mortality among elderly people. In spite of its recognized importance as pathogen, in Latin American countries including Colombia, there are not studies about the susceptibility or epidemiology of this bacterium. Therefore, the aim of this study was to determine the frequency of *C. difficile* Associated Disease in hospitalized patients and the susceptibility of *C. difficile* isolates to metronidazole-MTZ, vancomycin-VAN and clindamycin-CLM, in three tertiary hospitals of Medellín, Colombia from January 2013-January 2014. Stool samples were collected from patients with clinical symptoms that received antimicrobial therapy for more than 5 days. Samples were evaluated for toxin production using an enzyme immunoassay-EIA and cultured in Cefoxitin Cycloserine Fructose Agar medium. Antimicrobial susceptibility for MTZ and VAN was determined by agar dilution method and for CLM by Epsilometer test. The breakpoints for Minimum Inhibitory Concentrations-MICs were established according to CLSI criteria (document M100-23SE). A total of 405 stool samples were collected; most of patients were from hospital A (74%) and B (22%), both University hospitals, and the remaining 4% from C. The frequency of positive toxin was of 7% (28/405). We observed 26 patients with both toxin and culture positive, 32 with only positive culture and 3 with toxin positive and culture negative. A total of 63 isolates were obtained, all of them were susceptible to MTZ and VAN (MIC_{50} ranged from 0.64-16 µg/ml and 0.25-1 µg/ml, respectively), and 54% were resistant to CLM (MIC_{50} 16-256 µg/ml). These results indicate that *C. difficile* from these hospitals remains susceptible to the first choice antimicrobial therapy (MTZ or VAN). The level of CLM resistance could reflect the ample use of this anitmicrobial therapy for anaerobic infections. In those patients with culture positive and toxin negative could be due to the presence of non-toxigenic isolates, toxin degradation or technical limitations. Implementation of molecular or cytotoxicity assays could improve these results.

1245 ***POSTER SESSION III: CLOSTRIDIUM DIFFICILE BACTERIAL THERAPY***

Posters will be presented in Poster Session III
Tuesday, July 1, 1245-1345.

PIII-30

CHANGES IN THE GUT MICROBIOME FOLLOWING FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH RECURRENT *CLOSTRIDIUM DIFFICILE* INFECTION

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Clostridium difficile infection (CDI) is currently the leading cause of healthcare-associated diarrhea, costing up to $3B dollars annually. Of particular concern is the rising rate of recurrent CDI, which occurs in 20-30% of CDI patients. Within the recent decade, an alternative treatment method, fecal microbiota transplantation (FMT), has reemerged as an effective biotherapeutic method for recurrent CDI. Although prior studies have observed repopulation of the gut microbiota following FMT, how FMT specifically promotes disease resistance and further *C. difficile* colonization remains unknown. In this study, we investigated the changes in the gut microbiota following FMT in recurrent CDI patients. Using 16S rRNA analysis, we observed that the composition of the microbiota resembled that of the donor rather than the community before transplantation within the recipient. Although we observed variability among recipient-donor comparisons, the overall diversity of both Bacteroidetes and Firmicutes were increased post-FMT, whereas Proteobacteria levels were decreased. The clinical success rate of treatment was 92%. Because the functional profile of the gut environment may be more indicative of susceptible states than the community structure alone, we utilized PiCrust, a predictive metagenomic tool, to impute the metagenomic potential of the microbiome. We observed significant differences in the metagenome of samples before and after FMT. In particular, several amino acid transporter systems were overrepresented in samples collected prior to FMT. These results suggest that functional changes accompany microbial structural changes following this therapy. Future studies that incorporate the identification of specific functions, or metabolic states, that promote disease resistance rather than structure alone may be helpful in the identification of improved therapeutics for recurrent CDI.

***1245 POSTER SESSION III:* NEW INSIGHTS INTO *CLOSTRIDIUM DIFFICILE* PATHOGENESIS**

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Posters will be presented in Poster Session III
Tuesday, July 1, 1245-1345.

Posters will be presented in Poster Session III
Tuesday, July 1, 1245-1345.

CLOSTRIDIUM DIFFICILE: THE ROLE OF THE VEGETATIVE CELL IN PATHOGENESIS

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Clostridium difficile is a Gram positive, anaerobic, spore forming bacillus and is the leading cause of antibiotic associated diarrhoea (AAD) worldwide. *C. difficile* is becoming an ever increasing problem in healthcare environments and with reports of the few currently available treatments becoming more ineffective it is critical that *C. difficile* is researched in greater depth. It is known that the spore is essential for transmission of *C. difficile* and that the vegetative cell is essential for toxin production, which ultimately causes disease. However, it is not known whether the vegetative cell is merely a vessel for toxin production which begins to sporulate immediately after germination, or whether the vegetative cell in fact colonises and persists in the gut. The aim of this work is to investigate the role of the vegetative cell in the pathogenesis of *C. difficile* infection. A *spo0A* mutant, that is unable to sporulate, was selected as a potential model to study the biology of the vegetative cell. It was found that in batch cultures the *spo0A* mutant grew as well as wild type sporulating form of *C. difficile*. However, the *spo0A* mutant was not able to survive in a single-stage continuous flow *in vitro* gut model. Investigating this, it was shown that the *spo0A* mutant tolerated acidic environments (ranging from pH 4.0 - 6.0) less well than the wild type. To interrogate the mechanism of acid sensitivity, RT-PCR approaches have been used. The genes investigated included; *gpr*, *rkpK*, *grpE* and *spo0A*. The *spo0A* gene is highly expressed in the wild type throughout a 24 hour growth period concomitant with spore formation. Interestingly, in the mutant *spo0A* is expressed is low quantities until 21 hours when expression increases rapidly. These results suggest that while the *spo0A* mutant may be useful for some *in vitro* work, it is not robust enough for longer term studies. However, it may have provided an insight into how the vegetative cell behaves in pathogenesis, suggesting that it may only exist as a vegetative cell for a short period of time. This mutant may in fact be a good model for the vegetative cell but the concept of a vegetative cell model may be unrealistic due to the fact that sporulation is such a key aspect in *C. difficile*.

CLOSTRIDIUM DIFFICILE DIARRHEA: 027, HIGHER COUNTS, MORE TOXIN, MORE LACTOFERRIN

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C. difficile is the leading known cause of nosocomial antibiotic-associated diarrhea. Reports have linked ribotype 027 with worse outcomes, and worse outcomes with the presence of toxin. To identify potential effects of ribotype and microbial load, we generated quantitative culture and analyte data using 47 anonymous, unlinked and already existing clinical samples, each from the same area of southwest Virginia and each containing a toxigenic ribotype of *C. difficile*. No clinical information was collected. Two groups, liquid samples (Bristol stool chart 7, n=18, 44% were 027) and 027 samples (n=57% were liquid) had mean total (vegetative cells and spores) counts of ~10^5/g, about 10-fold higher than solid samples (Bristol stool chart 1,2 and 3) and non-027 samples. In 66% of liquid and 57% of 027 samples vegetative cells outnumbered spores; in only 15% of solid and 29% of non-027 samples did vegetative cells outnumber spores. In liquid samples the average levels of toxin A, toxin B and lactoferrin were respectively 78 ng/g, 122 ng/g and 250 µg/g. Levels in 027 samples were 165 ng/g, 187 ng/g and 373 µg/g. In solid samples the levels were lower, 54, ng/g 13 ng/g and 40 µg/g. They were significantly lower in non-27 samples, 35 ng/g*, 7 ng/g* and 91 µg/g* (*$p<0.05$). Semi-solid (Bristol stool chart 4, 5 and 6) analyte levels and counts were intermediate between those in liquid and solid samples. Overall, higher counts and vegetative growth were associated with higher levels of toxins, with 027, with liquid stool and with higher fecal lactoferrin. Our results suggest relationships during *C. difficile* diarrhea between stool consistency, toxin level, microbial burden, the relative abundance of vegetative cells, ribotype, and inflammation.

SIALIC ACID AND *CLOSTRIDIUM DIFFICILE*

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In the late 1980s KH Wilson (Duke University) proposed that antibiotics suppress the commensal flora, disrupt normal mucin metabolism, and create excesses of free sialic acid and N Acetylglucosamine that *C. difficile* can exploit and without which, it cannot colonize. Our aim was to reassess the role of sialic acid in *C. difficile* colonization of susceptible hosts. The mean level of free sialic acid in hamster feces 24 h after clindamycin (10 mg/kg) was nearly twice its pre-clindamycin level ($p=0.06$) but fell ($p<0.05$) to its pre-clindamycin level after dosing with 250 mg of fresh human feces in 1 mL of anaerobic diluent or with *C. difficile* 630 (10^3 spores in 1 mL). Hamsters receiving the fecal transplant were refractory to challenge with *C.difficile* spores; hamsters that did not receive the transplant were susceptible to colonization and diarrhea. As in hamsters, sialic acid levels in feces from healthy humans and from those with *C. difficile* diarrhea were similar, suggesting the nomal flora, *C. difficile* and an effective probiotic are competitors for the sialic acid. In a rtPCR study of select components of the normal flora of hamsters before and after clindamycin and *C. difficile*, the most significant finding was the fall in *Akkermansia mucinophila* levels to below detectable levels within 24 h of the antibiotic. Unlike the smaller and temporary falls seen with several other groups, including *Clostridium* XIVa which contains many mucin degraders, *A. mucinophila* levels did not recover. An *in vitro* assessment of growth, based on GDH, showed two ribotype 027 isolates of *C. difficile* produced twice the GDH growing on sialic acid than they did on glucose and other sugars. Probing online whole genome sequences of *C. difficile* for sialidase genes shows while other genes of the sialic acid operon are present, the sailidase gene is not, indicating it is the pool of free sialic acid produced by the residual flora, not mucin itself, that is critical to colonization. Once it has bound the free monomer, *C. difficile* grows well on sialic acid. We have confirmed that *C. difficile* colonization requires excess free sialic acid. Fecal transplants restore pre-antibiotic levels of sialic acid. This should be a requirement of effective probiotic treatments.

HIERARCHICAL EXPRESSION OF THE CODY REGULON IN *CLOSTRIDIUM DIFFICILE*

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CodY, a global regulatory protein that monitors nutrient sufficiency by responding to intracellular levels of the branched-chain amino acids and GTP, has been shown to regulate the expression of the principal toxin genes (*tcdA* and *tcdB*) as well as genes involved in amino acid biosynthesis, nutrient transport and fermentation pathways in *C. difficile*. In this study, our goal is to reveal the strategy used by *C. difficile* for integrating central metabolic pathways with virulence gene expression by determining the hierarchy of gene expression mediated by CodY. A combination of genetic and systems biology approaches is being used to determine the ranking of CodY target genes. Crystal structure studies of *B. subtilis* CodY ($CodY_{BS}$) showed that the N-terminal GAF domain contains a hydrophobic pocket with residues essential for full activation of CodY by the BCAAs. By creating point mutations in the CodY GAF domain, we are building a series of mutant strains with a range of residual CodY activities. To do so, we first modeled the *C. difficile* CodY ($CodY_{CD}$) GAF domain using Molrep, which highlighted major differences between the BCAAs binding pockets of $CodY_{BS}$ and $CodY_{CD}$, suggesting a rather different mode of activation of $CodY_{CD}$ by the BCAAs. Using site-directed mutagenesis, we have generated nine variants of $CodY_{CD}$ protein with single amino acid substitutions in the GAF domain. By using RNA-seq to compare the expression of all CodY target genes in the collection of *C. difficile codY* mutant strains, we will be able to determine the order in which the cell has evolved to prioritize regulation of each gene by CodY. Some of the mutant strains are expected to have residual levels of CodY activity similar to those characteristic of the wild-type strain under the nutrient-limiting conditions found in the colon.

PROTEOMIC ANALYSIS OF SURFACE PROTEINS FROM BRAZILIAN STRAINS OF *CLOSTRIDIUM DIFFICILE* TREATED WITH SUBINHIBITORY ANTIBIOTIC CONCENTRATIONS

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Clostridium difficile (Cdiff) is the etiological agent of antibiotic-associated diarrhea. When a susceptible individual is exposed to Cdiff, colonization starts primarily through interaction with surface-associated proteins (SP). Several studies have examined the impact of antibiotics in Cdiff toxins A and B production. However, not enough information is available about the interference of antibiotics in SP expression. The aim of this study was to evaluate the impact of sub inhibitory concentrations of two antibiotics, clindamycin and levofloxacin, on the expression of SP using three *C. difficile* strains isolated in Brazil. In addition, two strains, BI/NAP1/027 and 630, were used for comparison. MICs for clindamycin and levofloxacin were determined by E-test strips. The antibiotics were added to the Brucella broth to a final concentration of 0.5 x MIC, and the bacteria were grown overnight in an anaerobic cabinet at 37°C. Enriched fractions of SP were obtained by using low pH glycine incubation. Proteins were analyzed using SDS-PAGE gels after coomassie colloidal staining. All bands that presented apparent significant changes were excised from the gel and processed for further mass spectrometry analysis (nUPLC-MS/MS). SDS-PAGE gels suggested that clindamycin had a stronger effect in protein expression in the majority of the Cdiff strains examined. MS identification of modified bands revealed that cell wall binding protein (Cwp2), Cwp20, heat shock protein GroEL, S-layer protein (SlpA) and cell surface protein penicillin-binding protein (AmpC), were affected by clindamycin treatment. Our study brings novel information that will help to elucidate the involvement of the SP in the pathogenesis of *C. difficile*.

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CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* IN TOTAL STOOL DNA FROM CHILDREN BY DIRECT PCR-RIBOTYPING

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Direct PCR-ribotyping is a modified method which, due to increased specificity of primers, enables culture independent detection of *C. difficile* PCR-ribotypes. It is useful as a rapid genotyping method in cases when isolates are not available.

In this study altogether 382 children (1 month to 6 years; median age 20 m), with (295) and without (87) the symptoms of gastroenteritis, were screened for the presence of *C. difficile*. Only total stool DNA was available and hence no strains were isolated. *C. difficile* was detected by in-house real-time PCR specific for *C. difficile* 16S rRNA gene. For detection of toxigenic strains RT PCR targeting toxin genes *tcd*A and *tcd*B was used. All *C. difficile* positive samples were tested by direct PCR-ribotyping.

Fifty (13.1%) stool samples were positive by 16S RT PCR and in 29 (7.6%) samples toxin genes were detected. Five (5.7%) toxin genes positive samples were from children without the symptoms of gastroenteritis and 24 (8.1%) from children with the symptoms. Direct PCR-ribotyping was possible for 40 out of 50 *C. difficile* positive samples (80%). The remaining 10 *C. difficile* positive samples were either negative or represented weak fragments that could not be analyzed. These 10 samples also had weakly positive results on RT PCR (Ct 30 to 36). Overall, 23 different PCR-ribotypes were identified (002, 009, 010, 012, 014/020, 017, 018, 023, 029, 033, 053, 070, 150 and 10 types with internal lab designation only). In two samples indication of co-infection with pathogenic and non-pathogenic strain was observed.

Toxigenic PCR-ribotypes that are usually associated with *C. difficile* infections are also commonly found in children. Although children rarely develop a disease, they can be an important reservoir of pathogenic strains.

IDENTIFICATION OF IRON ACQUISITION MECHANISMS IN *CLOSTRIDIUM DIFFICILE*

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The bacterial pathogen *Clostridium difficile* remains a leading cause of nosocomial infection. *C. difficile* can colonize the gut after antibiotic therapy creates a favorable environment for its growth, causing intestinal disease ranging from diarrhea to severe colitis and death. Despite its significance in human health, few pathogenic mechanisms in *C. difficile* have been identified. In other bacteria, iron acquisition holds an important role in pathogenesis. Iron is a vital nutrient for most living organisms as it is a cofactor for enzymes involved in key cellular processes. However, free iron is limited in hosts, requiring bacteria to evolve specialized means to obtain the metal. This research seeks to identify the unknown mechanisms *C. difficile* employs to obtain iron. Global transcriptome analysis was conducted to identify changes in gene expression levels stimulated by iron-limited conditions. Many of the genetic operons that are induced under iron-limited conditions in *C. difficile* possess homology to proteins with known iron acquisition roles in other bacteria. Several of these operons were selected for further study; among the operons' hypothesized functions include predicted siderophore, ferrous iron, and polyamine transporters. To determine the role of these proteins, deletion constructs were created and transferred into the *C. difficile* 630 to produce mutants lacking these specific genes/operons. The resulting mutants were analyzed by growth kinetics under iron-starved conditions to evaluate the function of the deleted gene. Any iron acquisition mechanisms identified as essential will represent attractive targets for future therapeutic developments.

KINETICS OF *C. DIFFICILE* INFECTION THROUGHOUT THE GASTROINTESTINAL TRACT

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Background: *C. difficile* infection (CDI) is the most common cause of healthcare-associated diarrhea and colitis. CDI results in upwards of 14,000 deaths annually and $4.8 billion in excess healthcare costs. Work has been done to define *C. difficile* infection *in vivo* however no report has defined infection throughout the gastrointestinal (GI) tract to determine the kinetics of *C. difficile* germination, colonization, sporulation, toxin production, and disease progression. This study defines the longitudinal and temporal changes to the GI tract during *C. difficile* infection. Additionally we discuss the sites in the GI tract where germination and vegetative cell outgrowth can occur.

Methods: Five-nine week old C57BL/6 mice were given cefoperazone (0.5 mg/ml) in dH_20 for five days then given dH_20 for two days. Mice were challenged with *C. difficile* VPI10463 spores and mice (n=5 per group) were randomly euthanized and necropsied every six hours throughout infection for up to 36 hours. Luminal content at each GI site from the stomach to the rectum was collected and used to determine the level of *C. difficile* vegetative cells, toxin, spores and disease.

Results: Six hours after challenge *C. difficile* spores germinated and 10^3 vegetative cells were identified in the large intestine. At 18 hours post challenge 10^7 vegetative cells were present in the large intestine. Additionally there were no detectable spores or toxin throughout the GI tract. At 24 hours post challenge vegetative cells and spores were identified throughout the entire GI tract, with highest toxin levels in the large intestine. By 36 hours post infection mice became moribund and succumbed to infection.

Conclusions: Germination and colonization of *C. difficile* in the large intestine occurs quite rapidly after challenge with *C. difficile* spores. Disease progression occurs within 24 hours post challenge. These experiments will help define the location and environment where *C. difficile* germinate, grow, and produce toxin. This will help us better understand disease progression in order to design future therapeutics for prevention of CDI.

IMPACT OF *C. DIFFICILE* EXOSPORIUM ON RAMOPLANIN ACTIVITY IN AN *IN VITRO* MODEL OF SPORE PERSISTENCE

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Ramoplanin is a glycolipodepsipeptide antibiotic that is bactericidal for many gram-positive aerobic and anaerobic bacteria, including *C. difficile*. Ramoplanin has been reported to also have activity against *C. difficile* spores, both *in vitro* and in an animal model. We sought to evaluate whether this observation reflects direct sporicidal activity or a different mechanism. 10^5 *C. difficile* spores (ribotype 027) were incubated in 9 mL of chilled water containing ramoplanin at multiple concentrations above the MIC. Ramoplanin exposure was continuous for six days, and daily samples of exposed spores were enumerated anaerobically on blood agar supplemented with taurocholate after four consecutive washes and serial 10-fold dilutions. A separate preparation of spores was sonicated and enzyme treated to remove the exosporium components prior to ramoplanin exposure and washes. The persistence of ramoplanin activity was assessed via disk diffusion bioassay using *S. salivarius* as an indicator organism. Spores exposed to ramoplanin at 300 mg/mL could not be recovered at any time after exposure. Lower concentrations of ramoplanin exposure resulted in reduced recovery compared to control samples. Spores that had undergone enzymatic and sonication processing were also recoverable. Ramoplanin-exposed spores with intact exosporium inhibited growth of indicator organisms after multiple washings whereas exosporium-stripped spores did not. No difference in such activity was observed between 15 minutes and 6 days. Growth-inhibitory levels of ramoplanin remain on ramoplanin-exposed spores after multiple washes. This effect is muted by removal of the exosporium before exposure, implying that the exosporium may be the target for ramoplanin binding, permitting an "ambush" type of vegetative cell killing once the spores are plated. Direct sporicidal activity seems unlikely since spores that had undergone exosporium processing were still recoverable after ramoplanin exposure. The rapid and persistent activity of ramoplanin on *C. difficile* spores could have beneficial clinical effects in patients with recurrent *C. difficile* secondary to relapse.

THE EFFECT OF SMT19969 ON SPORE GERMINATION, OUTGROWTH AND SPORULATION IN *CLOSTRIDIUM DIFFICILE* 630

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Clostridium difficile infection is still a major cause of hospital acquired diarrhoea. It causes a substantial health and financial burden, a particular problem being recurrent disease. Treatment options are limited, with only metronidazole and vancomycin routinely used. Recent reports of strains less susceptible to metronidazole aggravate the situation. The new antibiotic fidaxomicin, which may not disturb the gut micro-flora as much as metronidazole and vancomycin, yields better success in terms of sustained cure, except for infections due to hypervirulent PCR-ribotype 027 strains where comparable recurrence rates are seen.

SMT1996 is a novel and promising non-absorbed antibiotic, which is more potent than vancomycin and metronidazole against *C. difficile* and shows minimal impact on the host gut micro-flora due to its very narrow spectrum of activity.

Here we evaluated the effect of SMT19969 on spore germination, outgrowth and sporulation using *C. difficile* strain 630.

Germination defines the loss of spore-specific characteristics, such as heat-resistance. Spore stocks of *C. difficile* 630 were prepared and spores incubated in BHIS or BHIS + SMT19969 (MIC = 0.0625 ug/mL) either with or without the germinant taurocholate (TC). A significant drop in colony forming units (cfus) was observed in all samples with TC (+/-SMT19969) between time point zero hours and one hour, showing that the spores were able to germinate. No effect on germination was recorded at any concentration of SMT19969 (½x Minimal Inhibitory Concentration (MIC) – 10x MIC).

Outgrowth is the process in which successfully germinated spores grow into vegetative cells. This was measured as an increase in OD_{600}. At ½x MIC and MIC outgrowth was comparable to wild-type, however at 5xMIC a delay was observed and there was no outgrowth at 10x MIC.

Finally sporulation was measured by plating heat resistant cfus at a range of time points onto BHIS + TC. A significant reduction (> 3 $\log_{10}$) in the number of spores produced was observed at SMT19969 concentrations of 5xMIC and higher when compared to drug free controls.

Overall SMT19969 appears to act on the vegetative cell and not on the spore. The reduction in sporulation may result in a reduced spore load in the GI tract of CDI patients and this effect, including comparison to vancomycin and fidaxomicin, is being investigated further.

IN VITRO EXPRESSION OF *CLOSTRIDIUM DIFFICILE* BINARY TOXIN

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Clostridium difficile is the world's leading known cause of nosocomial diarrhea. In addition to the two main virulence factors (Toxins A and B) several strains produce a third, binary toxin composed of two unlinked proteins designated CdtA and CdtB. Although the role of binary toxin is still unclear, it is gaining attention as a possible virulence factor. Our aim was to investigate the *in vitro* expression of CdtB across a panel of ribotypes that carry the genes for binary toxin. As controls, we included ribotypes that do not carry the *cdtB* gene. Brain-heart infusion broths were inoculated with stock *C. difficile* cultures and incubated 24 or 72 hours anaerobically at 37°C. Culture fluids were assayed in triplicate using an in-house monoclonal antibody-based ELISA to detect CdtB. We assayed a total of 33 isolates across 29 different ribotypes. Twenty two ribotypes carried genes for binary toxin, and 19 of these made detectable amounts of CdtB *in vitro*. All seven ribotypes that did not carry the genes for binary toxin were negative. As an extension of this study, we inoculated brain-heart infusion broths with 24 hour cultures of *C. difficile*. The broths were harvested after 0, 2, 4, 8, 16, and 24 hour anaerobic incubation at 37°C. Several ribotypes made detectable amounts of CdtB within 8 hours of growth. To summarize, we have an ELISA that can detect CdtB expressed by *C difficile in vitro*. Our data show that binary toxin is not a ribotype 027-specific phenomenon. Further investigation is needed to learn more about the role of binary toxin as a virulence factor of *C. difficile*.

TOXINS PRODUCED BY *CLOSTRIDIUM DIFFICILE* RIBOTYPE 027/NAP1 STRAINS CAN BE DIRECTLY DIFFERENTIATED THROUGH TARGETED PROTEOMICS

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Clostridium difficile (Cdiff) toxins (TcdA and TcdB) are part of a family of structurally and functionally related large clostridial exotoxins. Although the amino acid sequences of TcdA and TcdB are similar among Cdiff strains, consistent differences can be correlated to specific ribotypes. We report the direct differentiation of toxins produced by different types of Cdiff using a targeted proteomic approach on the secretome of clinical Cdiff strains. Tryptic digests of culture supernatants (CS) of toxin-producing Cdiff clinical strains belonging to ribotypes 027, 078 and 014 were analyzed using an array of proteomics technologies that incorporates protein separation methods, liquid chromatography-mass spectrometry (LC-MS), tandem mass spectrometry (MS/MS), multiple reaction monitoring (MRM) and bioinformatics. *In silico* analysis revealed unique toxin peptides that correlate to each ribotype and this was further confirmed after targeted proteomic analysis. Amino acid sequences of TcdA are 96-99% similar among Cdiff ribotypes but we identified 45 unique peptides in the CS of ribotype 027/NAP1 isolates. TcdB sequences are 92-98% similar and we identified 140 unique peptides that can discriminate 027/NAP1 strains. Further, analysis of CS tryptic digests by MRM using a triple quadrupole MS instrument confirmed direct differentiation of ribotype 027/NAP1 strains achieved by targeted toxin proteomics. These unique 027/NAP1 peptides may be useful for quantitative analysis of Cdiff toxins in biological samples by isotope-dilution MS (IDMS). In conclusion, targeted proteomic detection of Cdiff toxin peptides may result in the development of an IDMS method for Cdiff strain differentiation and toxin quantification that does not require DNA analysis. This method may be applied to clinical samples for improved diagnostics and understanding of Cdiff pathogenesis.

VIRULENCE POTENTIAL OF A-B+ *CLOSTRIDIUM DIFFICILE* STRAINS

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The main virulence factors responsible for *C. difficile*-induced diseases are toxin A and toxin B. The majority of toxigenic strains harbor both toxins; however, there is an important group of strains that do not produce toxin A. These are known as A-B+ strains and have been reported to produce a variant toxin B. Within a group of clinical isolates from a *C. difficile* outbreak, two strains lacking toxin A were identified. The aim of this work was to study the virulence potential of these A-B+ isolates.

One of the strains belongs to the NAP9 genotype whereas the other isolate belongs to a previously undescribed macrorestriction pattern. Only the latter encoded the *cdt* gene whereas none of them hyper produced toxin A or toxin B. The NAP9 strain was highly resistant to clindamycin, fluoroquinolones, and rifampicin. Mutations in *gyrA* and *gyrB* and *rpoB* are responsible for resistance to these antibiotics. The other strain was only resistant to ciprofloxacin. Cells intoxicated with purified toxin B from both strains were completely rounded and detached easily from the surface, resembling the effect induced by toxin B from toxin A-negative strains. The toxin B glucosylation patterns were determined on intact cells using pull down assays and *in vitro* using recombinant small GTPases. Whereas toxin B from classic nosocomial strains glucosylate Rho, Rac and Cdc42, toxin B from both Toxin A-negative strains did not modified Rho but did glucosylate Rap and R-Ras.

We are currently analyzing these variant strains in animal models and by whole genome sequencing to understand their virulence potential and the relative contribution of different molecular factor to the pathogenesis of *C. difficile*-induced diseases.

COMPARATIVE ANALYSIS OF *C. DIFFICILE* BACTERIAL PHENOTYPES AND VIRULENCE FACTORS BETWEEN CLINICAL ISOLATES OF SINGLE AND RECURRENT *CLOSTRIDIUM DIFFICILE* INFECTIONS

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Clostridium difficile is a major cause of nosocomial antibiotic-associated diarrhea in developed and developing countries. Recurrent *C. difficile* infections (rCDI) are primarily due to antibiotic-induced dysbiosis to an extent such that the colonic microbiota is unable to recover after the first episode of CDI. However, the contribution of bacterial virulence factors and several phenotypes (i.e., biofilm formation, spore adherence, and spore germination) to persistence of CDI remains poorly understood. In this work, *C. difficile* clinical isolates from Chilean patients with a single episode of CDI and recurrent CDI were analyzed for virulence factors and several bacterial phenotypes that might be involved in persistence of CDI. Our results demonstrate no difference in cytotoxicity, motility and sporulation between isolates from the single episode group and relapse group. The differentiation stage of intestinal epithelial Caco-2 cells did not affect adherence of spores of the relapse group. In contrast, spores of single episode group adhered to a lesser extent to undifferentiated Caco-2 cells than to differentiated Caco-2 cells. No difference in spore germination was observed between spores of the single episode and relapse episode group; taurocholate increased germination efficiency of spores independent of their clinical background. Isolates from the single episode group had similar biofilm formation as isolates of the relapse group; addition of glucose decreased biofilm formation of isolates of the relapse but not of the single episode group. In summary, our results suggest that the phenotypes related to toxin cytotoxicity, motility, sporulation, spore adherence to intestinal epithelial cells, germination and biofilm formation might not be major determinants in recurrence of CDI.

MOLECULAR EPIDEMIOLOGY OF HYPER-TOXIGENIC *CLOSTRIDIUM DIFFICILE* STRAINS IN SOUTHERN TAIWAN

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Clostridium difficile is the major cause of nosocomial diarrhea. Limited epidemiologic studies on *C. difficile* have been exploited in Taiwan. A total of 253 *C. difficile* isolates from different patients were collected between January 2011 and December 2012 in southern Taiwan. The goal of this study was to characterize pathogenicity locus diversity and antimicrobial susceptibility of these clinical isoaltes. We analyzed the prevalence of toxin A (*tcdA*), B (*tcdB*), and binary toxin genes (*cdt A/B*) and *tcdC* mutations in all isolates by multiplex-PCR. There was no *tcdA-negative, tcdB-positive C. difficile* in our collection. A total of 67.6% (171/253) of isolates harbored *tcdA* and *tcdB* (A^+B^+). Among these, twenty-one isolates (12.3%) were also positive for *cdtA/B*, which carry a *tcdC* gene deletion, including a 18-bp (19%) or 39-bp deletion (62%). These 21 hyper-toxigenic strains were further typed by PCR-ribotyping and multilocus sequence typing (MLST). There was no ribotype 027 and 001 in these isolates. The most frequent PCR-ribotypes were RT127 (27.3%), RT78/126 (22.7%), RT017 (14.2%), 034 (14.2%). Fifteen (71.4%) resistant isolates were identified, including thirteen moxifloxacin-resistant and five metronidazole-resistant strains. Three metronidazole-heteroresistant strains were identified. Here, we demonstrated an emerging drug resistance and predicted *tcdC* truncating mutations in a group of hyper-toxigenic *C. difficile* strains with both toxin A/B and binary toxin genes, suggesting that monitoring and surveillance for the existence of hyper-toxigenic strains in Taiwan is warranted for the controlling of further spreading.

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THE CYTOTOXICITY OF *CLOSTRIDIUM DIFFICILE* TOXIN B

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Gram-positive bacterium, *Clostridium difficile*, is responsible for a number of gastrointestinal diseases and is the major cause of antibiotic-associated diarrhea worldwide. Toxin A (TcdA) and toxin B (TcdB) are the main virulence factors secreted by *Clostridium difficile* to elicit its pathogenicity. It's largely unknown how these two toxins enter host cells although it's been suggested their internalizations are mediated by host receptors. Here we report the screening of host components important for TcdB toxicity, especially the identification of the toxin receptor, designated as TBR1 (TcdB Receptor 1). TBR1 was initially isolated from a whole-genome human shRNAmir library screening in HeLa cells. Using TALEN- and CRISPR-mediated gene knockout techniques, we demonstrated that the loss of TBR1 expression in HeLa cells significantly inhibited cell rounding and death caused by the treatment of TcdB, but not TcdA. In addition, the adventitious expression of TBR1 significantly sensitized human intestinal cells (HT-29 and Caco-2) to TcdB exposure. The direct interaction between TBR1 and TcdB has been confirmed, and the addition of the toxin-binding domain of TBR1 in the cell culture medium was able to protect cells from the toxic effects of TcdB. Our finding of the TcdB receptor reveals a previously unsuspected role for TBR1, and may provide a new therapeutic target for the treatment of *Clostridium difficile* infection. In addition, we have also conducted a large scale screening using CRISPR/Cas9 human knockout library for host components involved in TcdB toxicity. Results from this genetic screening will be reported.

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