Program & Abstract Book

Anaerobe 2012

The 11th Biennial Congress of the Anaerobe Society of the Americas

Grand Hyatt Hotel
San Francisco, CA USA
June 27-July 1, 2012

Program & Abstract Book

2012 Anaerobe

Contents

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Anaerobe 2012

Anaerobe Society of the Americas

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Welcome to **Anaerobe 2012**, the 11th biennial Congress of the Anaerobe Society of the Americas (ASA). This forum brings together clinicians and scientists from around the world to engage in presentations, dialogues, and interactions related to the clinical and microbiological aspects of anaerobic infections. The Congress will explore the role of anaerobes in both health and disease, while addressing the traditional and emerging technologies for identification and diagnosis.

Anaerobe 2012 again illustrates the international interest in the field of anaerobic bacteriology. The 158 papers included in this Abstract Book represent the work of over 515 scientists from 28 countries.

At this Congress, the Keynote Address will be given by **Dr. Peer Bork** of the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. Dr. Bork is a pioneer in novel approaches to the analysis of the microbiome to decipher its role in health and disease.

Lifetime Achievement Awards will be presented to **Dr. Elisabeth Nagy** of University of Szeged in Hungary and **Dr. Ian Poxton** of the University of Edinburgh in the United Kingdom. Both have contributed extensively to the field of anaerobic bacteriology, as researchers, educators, and organizational leaders.

I would like to thank the members of the Organizing Committee and the Session Chairs for their assistance in formulating what promises to be an exciting program. I also would like to thank those from industry—both patrons and exhibitors—for the financial support that makes this Congress possible, as well as grants from the U.S. National Institutes of Health, the European Society of Clinical Microbiology and Infectious Diseases, Burroughs Wellcome Fund, and the Gut Check Foundation.

In addition, I am grateful for our continued relationship with Anaerobe Systems for helping organize the expanded Pre-Congress Workshop and Microbiology Educational Services for providing the continuing education accreditation for laboratory scientists. And, 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 2012** serves to foster stimulating discussions as well as cultivate personal relationships that continue to invigorate the entire field beyond the timeframe of this Congress.

Cynthia L. Sears, M.D.
ASA President

About the Anaerobe Society

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

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

Anaerobe Society 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:

- National Institute of Allergy and Infectious Diseases
- European Society of Clinical Microbiology and Infectious Diseases
- Burroughs Wellcome Fund
- Gut Check Foundation

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- Sanofi Pasteur
- Shel Lab
- TechLab

Peer Bork, Ph.D.

Dr. Peer Bork is senior group leader and joint head of the Structural and Computational Biology unit at the European Molecular Biology Laboratory (EMBL), a European research organization with headquarters in Heidelberg, Germany. He also holds an appointment at the Max-Delbrueck-Center for Molecular Medicine in Berlin.

Dr. Bork received his Ph.D. in Biochemistry (1990) at the University of Leipzig and his Habilitation in Theoretical Biophysics (1995). He works in various areas of computational biology and systems analysis with a focus on function prediction, comparative analysis, and data integration. He says that he chose early on to combine his interest in computers and biology, because it provided him more room to develop his creativity than was typically open to a math-oriented student.

His team at EMBL focuses on microbes in two areas: ocean water and the human body. In the human intestine alone, there are 10,000 species of microbes that are unknown. Deciphering their behavior could help researchers address conditions such as diarrhea, which still causes a fifth of child deaths worldwide. "We must put the puzzle together on how these microbes interact and what risk factors we can correlate with them, such as diet or obesity. Today, you take antibiotics, and they kill pretty much everything. That's not healthy. We need to understand infections better, so we can come up with more clever ways to treat disease."

His group has published more than 500 research articles in international peer-reviewed journals. According to ISI (analyzing the last 10 years), Dr. Bork is currently the most cited European researcher in Molecular Biology and Genetics. According to Google Scholar, his most cited peer-reviewed papers are on the human genome, yeast proteome, mouse genome, and Simple Modular Architecture Research Tool (SMART). He has published on the human gut microbiome defining basic enterotypes and an interactive Tree of Life. He also serves on the editorial board of a number of journals, including *Science* and *PloS Biology*, and functions as senior editor of the journal *Molecular Systems Biology.*

Dr. Bork cofounded five biotech companies,two of which went public. More than 25 of his former associates now hold professorships or other group leader positions in prominent institutions all over the world. He received the *Nature Award for Creative Mentoring* for his achievements in nurturing and stimulating young scientists. He was also the recipient of the prestigious *Royal Society and Academie des Science Microsoft Award* for the advancement of science using computational methods and obtained a competitive *ERC Advanced Investigator Grant*.

In Memoriam

Susan Kinder Haake, D.M.D., Ph.D.
Dr. Susan Kinder Haake was a respected member of the UCLA School of Dentistry faculty in the sections of Periodontics and Oral Biology and Medicine, as well as a leading researcher on the role of oral bacteria in human health.

Born in Bath, ME, Dr. Haake earned her under-graduate degree from Hamilton College and a D.M.D. in 1979 from the Tufts School of Dental Medicine. She went on to pursue periodontal specialty training at the University of Connecticut, where she worked with Dr. Kenneth Kornman to pursue research on fastidious anaerobic bacteria and complete an M.S. degree in dental science in 1985.

When Dr. Kornman moved to the University of Texas Health Science Center in San Antonio, he recruited Dr. Haake to pursue graduate studies in microbiology. Earning a Ph.D. in 1993, she made a series of key discoveries about interbacterial adherence that underlie our current understanding about the central role of *Fusobacterium nucleatum* in the formation of dental plaque.

In 1991, Dr. Haake joined the faculty of UCLA's School of Dentistry. After establishing her own laboratory, she discovered a family of unique endogenous plasmids that she developed into the first shuttle vectors and other molecular tools for genetic manipulation of *F. nucleatum*. This work led to the identification of the outer membrane proteins that mediate the coaggregation of *F. nucleatum* with other oral bacteria.

Reflecting her broad perspective as both a microbiologist and periodontologist, she made a number of keen insights into the roles of periodontal bacteria in human health and disease. In recent years, Dr. Haake became a leading investigator in the NIH Human Microbiome Initiative. At the time of her death, she was heading an innovative, multidisciplinary project to elucidate the role of the periodontal microflora in gum disease.

Dr. Haake's numerous awards included the National Institutes of Health–National Institute of Dental Research *Physician-Scientist Award* (1987-1992); the *Robert B. Wolcott Award* of the Omicrom Kappa Upsilon Society (2004) for distinguished service to the dental profession; and diplomate status from the American Board of Periodontology (2007). In recognition for outstanding teaching and mentoring in periodontics, she received the American Academy of Periodontology's *Educator Award* (2008, 2012).

She died of pancreatic cancer at age 58 and is survived by her husband, Dr. David Haake; her children, Christine and Erik; two sisters; and two brothers.

Lifetime Achievement Award

Elisabeth Nagy, M.D., Ph.D., D.Sc.
Dr. Nagy is Professor of Clinical Microbiology at the University of Szeged Institute of Clinical Microbiology in Szeged Hungary. Born in Budapest in 1944, she received her M.D. from Albert Szent-Györgyi Medical University in 1968, as well as a Ph.D. (1983) and D.Sc. (1994) in clinical microbiology. She has taught Clinical Microbiology at University of Szeged for 41 years, including 14 years as Department Head.

Her current research interest is clinical bacteriology with particular emphasis on the clinically-significant anaerobic bacteria. Focus has been on *Clostridium difficile* infections, resistance mechanisms of Bacteroides strains against beta-lactam antibiotics, plasmid profile analysis of Bacteroides strains, antibiosis studies in connection with the ecological balance of the vagina and the small intestine, diagnostic problems of systemic fungal infections, and investigation of antifungal drugs. She has also been evaluating new diagnostic methods, such as sequencing and MALDI-TOF MS, in the routine diagnostics of anaerobic bacteria.

Dr. Nagy has been active in a variety of national and international organizations. She has been a Board Member of the Hungarian Society for Microbiology, the Hungarian Society for Chemotherapy, and Hungarian Society for Infectious Diseases and Clinical Microbiology, as well as serving as President of the Hungarian College of Clinical Microbiology. Over the past nine years, she has served the European Society for Clinical Microbiology and Infectious Diseases as member of the Executive Committee, Professional Affairs for Clinical Microbiology, and Chair of the ESCMID Study Group on Anaerobic Infections, since 2010.

She has authored 278 publications, with a cumulative impact factor of 197.471. She also has served as an editor of such journals as *Journal of Medical Microbiology* and *Clinical Microbiology and Infections*. Her awards include the Hungarian Society for Microbiology's *Manninger Medallion* (1995), the American Society for Microbiology's *Morrison Award* (1996), the Hungarian Society for Laboratory Medicine's *Pandy Medallion* (1997), and the Hungarian Society for Infectious Diseases and Clinical Microbiology's *Gerloczy Award* (2012).

Dr. Nagy is married, with three children and seven grandchildren. She has recently stepped down as Head of the Institute of Clinical Microbiology, but continues to work on research projects, including international collaborations.

Ian poxton, Ph.D., D.Sc.
Dr. Poxton is Professor of Microbial Infection and Immunity at the University of Edinburgh College of Medicine and Veterinary Medicine. Born in the north of England in 1949, he moved to Scotland in 1967 to be a microbiology student at the University of Edinburgh, where he has remained, except for a 3-year period of post doctoral work in Newcastle upon Tyne.

His current research interests include (1) the pathogenesis, immune response, molecular epidemiology and diagnosis of *Clostridium difficile* infections; (2) pathogenesis and vaccine development of diseases caused by other Clostridium species, notably Equine Grass Sickness; and in the recent past (3) the role of endotoxin/lipopolysaccharides in the host.

He has served two terms (2005-2009) as Chair of the European Society of Clinical Microbiology and Infectious Diseases' (ESCMID) Study Group for *Clostridium difficile* and served as Editor-in-Chief of the *Journal of Medical Microbiology* (2002-2007) and was Editor-in-Chief of *Reviews in Medical Microbiology* (1996-2002). He chaired the Society for Anaerobic Microbiology from 1999-2004.

Dr. Poxton has attended every meeting of the Anaerobe Society of the Americas (including the Buenos Aires one) since Marina del Rey in 1994 and was the first recipient of the *Sydney and Mary Finegold Award* at that first meeting. He has published almost 200 papers, including over 100 on anaerobes with nearly 50 on *Clostridium difficile.* He has co-authored two text books and has contributed chapters to many more. A new project, co-editing the second edition of *Molecular Medical Microbiology*, is just getting underway.

In his "spare time," birds, mountains, and wildlife photography are important. He has "birded" around the world with his wife Carol, seeing several thousand species in 45 or so different countries (including 430 spp from the contiguous States of the US). In the local Scottish hills, he continues a long-term study of the breeding biology of the Merlin (*Falco columbarius*), currently in its 29th year, and contributes to the ringing (US=banding) programme of the British Trust for Ornithology. To date, he has published 11 papers/articles in the ornithological press. He also has to get to the top of the last 52 of the "Munros": the 283 official Scottish mountains. These pastimes will continue as long as possible during imminent retirement.

Anaerobe 2012 Accreditation/Goals & Objectives

Anaerobe 2012—the 11th biennial Congress of the Anaerobe Society of the Americas—provides the forum for vigorous discussions of both the clinical and microbiological aspects of anaerobic infections, their diagnosis, and their therapy among medical practitioners, researchers, and laboratory scientists.

Physician Accreditation

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

Clinical Laboratory Scientist Accreditation

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

Microbiology Educational Services designates this educational activity for a maximum of 19 continuing education contact hours upon completion of the program and 14.0 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 by the Lunch Break on Sunday, July 1. Certificates can be picked up at the Registration Table, when your Evaluation Form is turned in at the conclusion of the Congress.

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 xxi-xxii.

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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Wednesday-Thursday June 27-28

800 Pre-Congress Workshop Registration Opens at Santa Clara University

900-1700 **ANAEROBE IDENTIFICATION WORKSHOP**
Santa Clara University, Santa Clara, CA

Thursday June 28

1200-1700 **CONGRESS REGISTRATION OPENS**
Grand Hyatt, San Francisco, CA

Friday June 29

700 **REGISTRATION / BREAKFAST / EXHIBITS**

830-840 **WELCOME REMARKS**
Cynthia L. Sears, M.D.
President, ASA

840-940 **SESSION I: KEYNOTE ADDRESS**

SI-1 Systemic Analysis of Human-Associated Microbes: Lessons from a Tiny Bacterium and a Large Community
Peer Bork, Ph.D.

940-955 **DISCUSSION**

955-1015 **BREAK / EXHIBITS**

1015-1145 **SESSION II: GASTROINTESTINAL MICROBIOTA & DISEASE**
Moderator: *Cynthia L. Sears, M.D.*

SII-1 Diet, the Human Gut Microbiome, and IBD
Gary D. Wu, M.D.

SII-2 The Microbiota and Inflammatory Bowel Disease: Insights from Disease Models
Deanna D. Nguyen, M.D.

SII-3 The Association of Fusobacteria with Colorectal Carcinoma
Robert A. Holt, Ph.D.

1145-1315 **LUNCH / EXHIBITS / COMMITTEE MEETINGS**
STUDENT COMPETITION PRESENTATIONS

1215-1315 ***C. DIFFICILE* MODEL WORKSHOP**

1315-1345

SESSION III: STATE OF THE ART LECTURE I

SIII-1 Cultivating the Uncultivable: How Can We Make Progress?
Slava Epstein, Ph.D.

1345-1445

SESSION IV: ORAL MICROBIOTA & DISEASE
Moderator: *Purnima S. Kumar, D.D.S.*

SIV-1 Oral Microbiota & Emerging Disease Associations
Emmanuel F. Mongodin, Ph.D.

SIV-2 The Links Between the Oral Microbiota & Pulmonary Disease
Frank A. Scannapieco, M.D.

1445-1545 POSTER SESSION I / EXHIBITS

1545-1635

SESSION V: ORAL PRESENTATIONS: MICROBIOTA IN HEALTH & DISEASE
Moderator: *Sidney M. Finegold, M.D.*

SV-1 Identification and Characterization of Bacterial Biofilms of Sporadic Colorectal Cancer
Christine Dejea

SV-2 Isolation of Novel, Non-*Bacteroides fragilis* Strains Containing the *Bacteroides fragilis* Toxin (Bft) Locus from Colorectal Cancer Patients
Andrew Goodwin, Ph.D.

SV-3 Human Intestinal Microbial Ecologies of Indigenous Communities
Paul A. Lawson, Ph.D.

SV-4 Gut Microbiota in Obesity: Preliminary Results
Gayane Martirosian, M.D., Ph.D.

1635-1735

SESSION VI: SCIENTIFIC FORUM: WHAT'S HOT

SVI-1 What's Hot in the Anaerobic Literature: *Bacteroides*
Bennett Lorber, M.D.

SVI-2 What's Hot in the Anaerobe Literature: *Clostridium difficile* Infections
Carl Erik Nord, M.D.

1735-1800

ANAEROBE SOCIETY MEMBERSHIP MEETING

1800-1900

WINE & CHEESE RECEPTION

Saturday June 30

700 **REGISTRATION / BREAKFAST / EXHIBITS**

800-845 **SESSION VII: STATE OF THE ART LECTURE II**

SVII-1 Vaginal Health, Ecology & Disease
Jeanne M. Marrazzo, M.D.

845-945 POSTER SESSION II / EXHIBITS

945-1045 **SESSION VIII: THE GENITAL MICROBIOTA**
Moderator: *David N. Fredricks, M.D.*

SVIII-1 Clostridium Vaginal Infections
Brandi Limbago, Ph.D.

SVIII-2 Anaerobes in Pelvic Inflammatory Disease: An Update
Sharon L. Hillier

1045-1100 **DISCUSSION**

1100-1145 **SESSION IX: STATE OF THE ART LECTURE III**

SIX-1 Fidaxomicin & New Agents in the Treatment of *Clostridium difficile* Recurrences
Thomas J. Louie, M.D.

1145-1200 **DISCUSSION**

1200-1315 LUNCH / EXHIBITS

1230-1315 *ANAEROBE* JOURNAL AUTHORSHIP WORKSHOP

1315-1445 **SESSION X: *CLOSTRIDIUM DIFFICILE*: PATHOGENESIS, EPIDEMIOLOGY & THERAPY**
Moderator: *Dale M. Gerding, M.D.*

SX-1 Development of a Murine Infection Model to Study *Clostridium difficile* Disease and Transmission
Trevor D. Lawley, Ph.D.

SX-2 Community-Associated *Clostridium difficile*: How Real Is It?
Fernanda Lessa, M.D., M.P.H.

Point-Counterpoint: *Clostridium difficile*: Is it a Food-borne Disease

SX-3 *Clostridium difficile* in Food: Supermarket Reality or Laboratory Contaminant?
Jane Marsh, Ph.D.

SX-4 *Clostridium difficile* is an Agent of Foodborne Disease
J. Glen Songer, Ph.D.

1445-1500 BREAK / EXHIBITS

SESSION XI: ORAL PRESENTATIONS: *CLOSTRIDIUM DIFFICILE*

Moderator: *Stuart Johnson, M.D.*

SXI-1 The Changing Faces of *Clostridium difficile*: A Personal Reflection of the Past 34 Years
Ian R. Poxton, Ph.D.

SXI-2 Colonization Resistance: Interactions Between *Clostridium difficile* and the Indigenous Gut Microbiota
Vincent B. Young, M.D.

SXI-3 Epidemiology of Community-Associated *Clostridium difficile* Infection (CA-CDI), Emerging Infections Program, 2009–2011
Amit Chitnis, M.D.

SXI-4 The Importance of Toxin A in Virulence of an Epidemic *Clostridium difficile* Strain
Sarah A. Kuehne, Ph.D.

SXI-5 Phenotypic Characterization of *Clostridium difficile* Clinical Isolates
Paul E. Carlson Jr., Ph.D.

SXI-6 Severe *Clostridium difficile* Associated Diarrhea—Impact of High Dose Vancomycin Therapy
Marina C. Claros, Ph.D.

1500-1630

SESSION XII: CLINICAL FORUM: UNKNOWN CASES

Panelists:
Gonzalo Ballon-Landa, M.D.
Ellen Jo Baron, Ph.D.
Ellie J.C. Goldstein, M.D.

1630-1730

CONGRESS BANQUET RECEPTION
NEIMAN-MARCUS ROTUNDA

1800-1900

CONGRESS BANQUET & AWARDS
NEIMAN-MARCUS ROTUNDA
FINEGOLD AWARD
YOUNG INVESTIGATORS AWARDS
LIFETIME ACHIEVEMENT AWARDS:
Dr. Elisabeth Nagy of Szeged Hungary,
Dr. Ian Poxton of Edinburgh, Scotland UK

1900

Sunday July 1

730 **REGISTRATION / BREAKFAST / EXHIBITS**

815-905 **SESSION XIII: STATE OF THE ART LECTURE IV**

SXIII-1 Anaerobic Diagnostic Microbiology: *Clostridium difficile* and Beyond
Diane M. Citron

SXIII-2 Is MALDI-TOF MS Superior to Commercially Available Identification Kits for Identification of Anaerobic Bacteria in Routine Laboratories
Elisabeth Nagy, Ph.D.

905-915 **DISCUSSION**

915-1000 POSTER SESSION III / EXHIBITS

1000-1110 **SESSION XIV: OTHER CLOSTRIDIAL INFECTIONS**
Sponsored by the Gut Check Foundation
Moderator: *Dennis L. Stevens, M.D., Ph.D.*

SXIV-1 Botulism: Newer Aspects
Stephen S. Arnon, M.D.

SXIV-2 *Clostridium* spp.: Mechanisms of Disease
David M. Aronoff, M.D.

1110-1200 **SESSION XV: ORAL PRESENTATIONS: *CLOSTRIDIUM SPP.***
Moderator: *J. Glen Songer, M.D.*

SXV-1 Recent Progress in Understanding the Action of *Clostridium perfringens* Enterotoxin
Bruce A. McClane, Ph.D.

SXV-2 Evidence that the Agr Quorum Sensing System is a Regulator of *Clostridium perfringens* Toxin Production and Virulence
Jianming Chen, Ph.D.

SVX-3 Highly Sensitive Cell Model for Botulinum Neurotoxin Detection Using Human Neurons from Induced Pluripotent Stem Cells
Eric A. Johnson, Ph.D.

SVX-4 Phenotype Microarrays and Cytotoxicity-Based Clostridial Toxin Assay
Xiang He Lei, Ph.D.

1200-1315 LUNCH / EXHIBITS

Sunday July 1

1315-1445

SESSION XVI: PROBIOTICS: MECHANISMS & HEALTH BENEFITS

Moderator*: Andrew B. Onderdonk, Ph.D.*

SXVI-1 Probiotics in Vaginal Infection Prevention
Raina N. Fichorova, M.D.

SXVI-2 Antibiotic-Associated Diarrhea: Are Probiotics the Answer?
Mark Wilks, M.D. / Jasmine Islam

SXVI-3 Probiotic Mechanisms of Action—One Bug's Story
D. Brent Polk, M.D.

1445-1545

SESSION XVII: ORAL PRESENTATIONS

Moderator*: Bennett Lorber, M.D.*

SXVII-1 Geographic Signatures of Anaerobes to Study Human-Microbe Co-Migration and Co-Evolution
Georg Conrads, Ph.D.

SXVII-2 Probiotics in the Control of the Incidence and Severity of *Clostridium difficile* Infections at a Community Hospital
Pierre-Jean Maziade, M.D.

SXVII-3 Polysaccharide Biosynthesis Loci Diversity amongst *Bacteroides fragilis* Isolates
Sheila Patrick, Ph.D., D.Sc.

SXVII-4 A Unique Homologue of Eukaryotic Ubiquitin Produced by *Bacteroides fragilis* with the Potential for Significant Host Interaction
Kelly J. Jobling

SXVII-5 Effect of Antiretroviral Treatment on Selected Gut Microbiota in South African HIV Positive Patients
Valerie R. Abratt, Ph.D.

SXVII-6 Gut Microbiota in Preterm Infants Throughout the First Year of Life
Marie-Jose Butel, Pharm. D., Ph.D.

1545-1600

DISCUSSION & CLOSING REMARKS

Cynthia L. Sears, M.D.

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Mike Cox	Anaerobe Systems (E, O)
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Sharon L. Hillier, M.D.	Merck (C)
Eric A. Johnson, Ph.D.	Cellsnap (O), Eric A. Johnson Consulting (C)
Stuart Johnson, M.D.	Astellas (C), BioK+ (C), Cubist (C), Optimer (C), Pfizer (C), ViroPharma (C)
Xiang He Lei, Ph.D.	Biolog (E) Research presented involves applications of Biolog products
Jeanne M. Marrazzo, M.D.	Graceway (C), StarPharma, (C), Toltec Pharmaceuticals (C)
Jane Marsh, Ph.D.	Merck (G), Viropharma, (G)
Pierre-Jean Maziade, M.D.	BioK + (A)
Ian R. Poxton, Ph.D.	Astellas (C), Merck (C), Optimer (C), ViroPharma (C, G)
Mark Wilks, M.D.	Danone (G) Will discuss several probiotic products
Vincent B. Young, M.D., Ph.D.	Viropharma (A)

Relationship: (A) Advisory Board, (C) Consultant, (E) Employment, (G) Grant, (L) Licensed Patents, (O) Ownership/Stock

Oral Abstract Contents

Anaerobe 2012

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

*Indicates Presenter

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

840 *SESSION I:* KEYNOTE ADDRESS

SI-1

SYSTEMIC ANALYSIS OF HUMAN-ASSOCIATED MICROBES: LESSONS FROM A TINY BACTERIUM AND A LARGE COMMUNITY

Bork, P.*
European Molecular Biology Lab, Heidelberg, Germany

Bacteria share many more molecular features with eukaroytic cells than currently appreciated and are convenient models for the study of many fundamental biomolecular processes. I will illustrate the power of such models using one of the smallest bacteria, *Mycoplasma pneumoniae*. Data on the transcriptome, metabolome and proteome have been consistently generated and integrated to reveal a wealth of information about the biology of a genome-reduced bacterium that was found to be remarkably complex (Kuehner, 2009, Yus, 2009, Guell, 2009, Van Noort, 2012). In order to utilize bacteria for human health, yet another layer of complexity has to be understood, that of the interactions of many bacteria forming microbial communities. The recent advent of environmental sequencing (metagenomics) enabled the collection of genomic parts lists of various microbial communities, but our understanding of their functioning still remains limited.

Using the human gut as an example, I will briefly introduce into recent technological advances (Qin, 2010) and will describe recent findings on the stratification of such communities in the human population with likely impact on personalized medicine and nutrition recommendations. For example, i) the microbial communities of each human individual can be classified into a few enterotypes (Arumugam, 2011) that are likely to respond differently to diet and drug intake and ii) each individual appears to carry a unique set of strains leading to individual genomic variation patterns with a likely individual antibiotic resistance repertoire.

Kuehner S *et. al.*, *Science*. 2009 Nov 27;326(5957):1235-40.
Yus E *et. al., Science.* 2009 Nov 27;326(5957):1263-8.
Guell M *et. al., Science*. 2009 Nov 27;326(5957):1268-71.
Van Noort V *et. al., Mol.Sys.Biol*. 2012, in press
Qin J *et. al., Nature*. 2010 Mar 4;464(7285):59-65.
Arumugam M *et. al., Nature*. 2011 May 12;473(7346):174-80.

1015 SESSION II: GASTROINTESTINAL MICROBIOTA & DISEASE

SII-1

DIET, THE HUMAN GUT MICROBIOME, AND IBD

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Division of Gastroenterology, Perelman School of Medicine
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We coexist with our gut microbiota as mutualists, but this relationship sometimes becomes pathological, as in obesity, diabetes, atherosclerosis, and inflammatory bowel diseases. Factors including age, genetics, and diet may influence microbiome composition. Of these, diet is easiest to modify and presents the simplest route for therapeutic intervention. In studies to be presented, we used diet inventories and 16S rRNA gene sequencing to characterize fecal samples from 98 individuals. Fecal communities clustered into enterotypes distinguished primarily by levels of *Bacteroides* and *Prevotella*. Enterotypes were strongly associated with long-term diets, particularly protein and animal fat (*Bacteroides*) versus carbohydrates (*Prevotella*). A controlled-feeding study of 10 subjects showed that microbiome composition changed detectably within 24 hours of initiating a high fat/low fiber or low fat/high fiber diet, but that enterotype identity remained stable during the 10-day study. Thus, alternative enterotype states are associated with long-term diet. Having demonstrated the impact of diet on human gut microbiome, we are now focusing on the impact of a dietary intervention known to be effective in the treatment of Crohn's disease, namely a defined formula diet, on the composition of the human gut microbiome. It is hoped that the results of these studies may help to identify bacterial taxa that play a role in the pathogenesis of Crohn's disease and/or serve as biomarkers that may help to predict response to therapeutic interventions.

THE MICROBIOTA AND INFLAMMATORY BOWEL DISEASE: INSIGHTS FROM DISEASE MODELS

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[1]Gastrointestinal Unit and Center for the Study of Inflammatory Bowel Disease, Massachusetts General Hospital, Boston, MA USA
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Inflammatory bowel disease (IBD) is thought to result from a dysregulated immune response to intestinal microbial flora in individuals with genetic predisposition(s). Genome wide association studies (GWAS) in human IBD have identified almost 200 associated loci, some of which are key players in innate immunity and bacterial handling, reflecting the importance of the microbiota in disease pathogenesis. In fact, the presence of a microbial flora is not only crucial to the development of a normal murine immune system, but also critical for the development of disease in the majority of animal models of IBD.

Although animal models do not perfectly recapitulate human IBD, they have led to the discovery of important concepts in IBD pathogenesis, such as the central role of microbiota in disease development and perpetuation. Many genetically susceptible models do not develop colitis when raised in a germ-free or *Helicobacter*-free environment. In fact, disease in most models can be attenuated or completely abolished with antibiotic treatment. Moreover, an interplay between intestinal bacteria and mucosal immune activation is suggested by the presence of serum antibodies against the Cbir1 flagellin, an immunodominant antigen that activates TLR5, in certain models of spontaneous colitis as well as in human patients. Furthermore, T cells reactive to Cbir1 are able to induce disease in recipient mice upon adoptive cell transfer, demonstrating the pro-inflammatory properties of certain bacterial products. In fact, it has been shown that transfer of certain bacteria from a specific genetically altered mouse model with spontaneous colitis can induce disease in wild-type mice upon co-housing. These observations demonstrate the pathogenic potential of intestinal microbiota in IBD.

However, intestinal bacteria are not always maladaptive in mucosal homeostasis. Polysaccharide A from *Bacteroides fragilis* reduces inflammatory cytokine production and protects against murine colitis. Along the same line, *Clostridium* species promote the number and function of regulatory T cells in the colon. In fact, normal development of regulatory cells and epithelial cell integrity are abolished in the absence of an intestinal flora, suggestive of the need for certain microbial components to induce beneficial anti-inflammatory mechanisms.

All in all, altered immune responses to microbes play a crucial role in IBD pathogenesis. However, certain components of the microbiota are also likely critical for normal development of regulatory mechanisms that contribute to mucosal homeostasis. Findings in animal models highlight the concept that IBD is a disease that results from the interplay of genetics and microbial/environmental factors.

SII-3

THE ASSOCIATION OF FUSOBACTERIA WITH COLORECTAL CARCINOMA

Holt, R.A.;*[1,2] Warren, R.L.;[1] Freeman, D.J.;[1] Watson, P.;[4] Moore, R.A.;[1,3] Cochrane, K.;[5] Allen-Vercoe, E.[5]
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Numerous cancers have been linked to infectious agents. Given that colorectal cancer is a leading cause of cancer deaths and the colon is continuously exposed to a high diversity of microbes, we compared the microbiome of colorectal tumors to matched normal control tissue from 11 patients, for the purpose of searching for tumor associated microbes. Using a metagenomics approach that involved RNA-Seq with host sequence subtraction, we detected and subsequently verified high over-representation *Fusobacterium* spp. in tumor relative to control tissue, and we verified this over-representation by qPCR in a larger cohort. Fusobacteria isolated from tumor showed unique genome content relative to *Fusobacterium* spp. reference sequences, and had the ability to invade gut epithelial cells in culture, as previously reported. More recently, we completed full metagenomic analysis of a set of 65 colorectal tumor and matched control samples, and we observed additional, though less abundant, anaerobic bacteria of the genera Leptotrichia and Campylobacter that significantly co-occur with *Fusobacterium* spp. in tumor specimens. Thus, we are seeing a pattern of tumor associated anaerobic microbes that suggests a possible oral origin. The presence of these bacteria was also associated with differential expression of several host oncogoenes and immune response genes, but it remains uncertain whether these bacteria have any etiological role, direct or indirect, in colorectal carcinoma.

1315 SESSION III: STATE OF THE ART LECTURE I

SIII-1 Cultivating the Uncultivable: How Can We Make Progress? 10
*Epstein, S.**

SIII-1

CULTIVATING THE UNCULTIVABLE: HOW CAN WE MAKE PROGRESS?

Epstein, S.*
Northeastern University, Boston, MA USA

Overwhelming part of microbial diversity in nature remains uncultivated and unexplored. This is so because traditional cultivation approaches allow access to a tiny minority of microbial species. Over the past decade we have developed several alternative approaches that significantly depart from the standard techniques. One such method is based on placing microorganisms into a diffusion chamber, and returning the chamber into the natural environment from where the cells were originally collected. This provides target species with the growth factors of their natural environment, allowing for growth of species whose metabolic requirements are unknown. A proof-of-concept experiment showed a 300-fold higher recovery rate of marine microorganisms over parallel cultures that utilized conventional Petri dish approach. Further experimentation showed the species composition of the isolates was also markedly different between the chamber and Petri dish. Miniaturization of the diffusion chamber allowed application of this method to isolation of human microbiota and led to isolation of dozen of novel strains from the human oral cavity and vagina. Another approach we have employed is based of separation of microbial cells and their cultivation in isolation from each other. This allows access to slower growing and rarer species whose growth in standard Petri dishes or enrichment cultures is often masked or inhibited by fast growing "weed" strains. We have applied these and other novel as well traditional methods to a variety of environments, from pristine marine and soil habitats to contaminated subsurface and human body. This presentation will focus on the results of our cultivation efforts, and principal lessons learned. The new methods appear markedly different in two ways. First, they lead to isolation of a much larger diversity of microbial species than do the standard techniques. Second, they produce cultures of microorganisms that are native to and representative of the target habitats, often matching species previously known only by their molecular signatures. We show that all these methods likely have biases, and produce unique culture collections. Therefore, the combination of methods is more efficient in recovering microbial diversity than any given single approach, and is therefore a method of choice in microbial discovery.

1345 *SESSION IV:* **ORAL MICROBIOTA**

SIV-1

ORAL MICROBIOTA & EMERGING DISEASE ASSOCIATIONS

Mongodin, E.F.*
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The human body is host to a myriad of organisms, collectively known as the human microbiota, which is essential to maintain health. The oral microbiota is a major component of the human oral cavity and is unique in its ability to promote oral health and disease, but also in its potential to induce other diseases, such as systemic diseases or even cancer. The oral microbiota is a complex community of over 800 bacterial species that exist in a dynamic and intimate relationship with the oral surfaces of the human host. Metagenomic approaches have recently been applied to the study of the oral microbiota, in order to understand its role within the oral cavity and analyze its fundamental characteristics and dynamics. The results from these studies have strengthened the belief that progression from oral health to disease is associated with significant shifts in the community structure of the oral microbiota and the emergence of potential oral pathogens. However, the features defining the healthy microbiome are still not completely understood, and it is still unclear whether or not healthy and diseased oral microbiomes are truly distinct. I will present evidence from ongoing studies that defining the healthy oral microbiome and its role in various diseases might not be as simple as previously thought. Multi-faceted systems biology strategies are needed to unambiguously define the characteristics of the oral microbiome and the associations with health and diseases.

LINKS BETWEEN THE ORAL MICROBIOTA AND PULMONARY DISEASE

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Associations between poor oral health and pulmonary diseases are possible through a variety of mechanisms. This presentation will provide an overview of the latest information on the problems of aspiration pneumonia and nosocomial-acquired pneumonia, especially ventilator-associated pneumonia (VAP). Both infections lead to serious consequences for morbidity and mortality in institutional setting. Results from a recent randomized trial that tested the effects of the oral topical antimicrobial chlorhexidine on the oral flora of ventilated patients will be presented. Biological mechanisms involving the oral microflora and host response that influence pneumonia will be discussed. Practical advice on how to translate this information into everyday practice will be presented.

Anaerobe 2012

1545 SESSION V: ORAL PRESENTATIONS: MICROBIOTA IN HEALTH & DISEASE

SV-1

IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL BIOFILMS OF SPORADIC COLORECTAL CANCER

Dejea, C.;*[1] Wick, E.;[2,3] Hechenbleikner, E.;[2] Romans-Judge, K.;[3] Peterson, S.;[4] Snesrud, E.;[4] Sears, C.L.[1,3,5]
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The human colon contains the largest most diverse bacterial population of any colonized area of the human body, and this community is now viewed as essential for host nutrition and mucosal immunity. While certain members of the colonic microbiota appear to promote the host's health, accumulating evidence has associated intestinal bacteria with disease initiation and progression in inflammatory bowel diseases (IBD) and colorectal cancer (CRC). The goal of our prospective study is to define in CRC patients if the tumor-adherent microbiota differs in composition, spatial organization, and mucosal interaction from microbiota adherent to normal tissue. Tissue samples were collected from CRC resection, as well as routine colonoscopy for analysis by fluorescent-in-situ hybridization (FISH), scanning electron microscopy (SEM), and microbiome 454 pyrosequence analysis. Massive bacterial invasion of the mucosal mucus barrier, constituting a biofilm of greater than 10^{10} bacteria/ml, was detected by a universal FISH probe on 100% (11/11) of right colon tumors, while bacteria were absent in the mucus of left colon tumors (11/11) as well as normal control biopsies (14/14). SEM analysis confirmed this finding, revealing the presence of biofilms on right tumors that were comprised of both mixed (3/6) and homogenous (3/6) bacterial morphologies. Laser capture microscopy was utilized to specifically select this biofilm population for sequence analysis. Preliminary sequence data suggests that the bacteria comprising these tightly adherent right CRC biofilm communities are conserved between tumors and unique from microbiome analysis of the whole tumor mucosal surface, and furthermore, these tumor microbiota populations (adherent and total) differ from normal flanking tissues within the same individual. In conclusion, we have identified a unique bacterial biofilm population associated with right, but not left-sided, CRC suggesting that the putative bacterial initiation of right and left-sided CRC differs.

ISOLATION OF NOVEL, NON-*BACTEROIDES FRAGILIS* STRAINS CONTAINING THE *BACTEROIDES FRAGILIS* TOXIN (BFT) LOCUS FROM COLORECTAL CANCER PATIENTS

Goodwin, A.;*[1] Badani, R.;[2] Hechenbleikner, E.;[3] Chen, L.;[1] Ellis, B.;[4] Romans-Judge, K.;[2] Pardoll, D.;[1,2,4] Carroll, K.;[1,4] Wick, E.;[2] Sears, C.L.[1,2]
Departments of [1]Medicine, [2]Oncology, [3]Surgery, and [4]Pathology,
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Enterotoxigenic *Bacteroides fragilis* (ETBF) are a subgroup of enteric bacteria that produce the metalloprotease *B. fragilis* toxin (BFT), cause rapid and persistent intestinal inflammation in wild-type mice, and induce large numbers of colon tumors in a mouse model of colorectal cancer (CRC). ETBF is the etiologic agent of diarrheal diseases in livestock and humans and small studies have associated ETBF carriage with inflammatory bowel diseases (IBD) and CRC. A prospective study is ongoing to isolate and characterize ETBF strains from tissue samples obtained at CRC surgical resection as well as routine colonoscopy. To date, ETBF has been detected (based on *bft* locus amplification) in 100% (11/11) and 73% (8/11) of left- and right-sided colon CRC patients, respectively, compared to 75% (12/16) and 58% (7/12) of left and right colon control samples. Perhaps due to improved sample handling, culture techniques, and screening PCR assay refinements, these prevalence rates are significantly higher than previously reported and warrant further investigation. More importantly, we report the discovery that numerous, non-*Bacteroides fragilis* species harbor the *bft* locus and, therefore, represent potential novel anaerobic pathogens in the etiology of diarrheal diseases, IBD, and CRC. The *bft* gene has been detected in at least eleven closely related species to date, and *bft*-encoding *B. vulgatus* appear to be about as prevalent as canonical ETBF. Further, 12 of 22 patients analyzed (including CRC and controls as described above, as well as IBD and familial adenomatous polyposis patients) are colonized with *bft*-positive strains of multiple species and numerous patients harbor ETBF/ETBF-like strains encoding more than one of the three molecular subtypes of *bft*. In conclusion, we have characterized a far more diverse group of ETBF-like organisms than previously described, presenting the possibility that certain subgroups may preferentially contribute to the etiology of IBD and CRC.

SV-3

HUMAN INTESTINAL MICROBIAL ECOLOGIES OF INDIGENOUS COMMUNITIES

Lawson, P.A.;*[1] O'Neal, L.;[1] Tito, R.Y.;[2,4] Obregón-Tito, A.J;[2,4]
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Guija-Poma, E.;[4] Lewis Jr., C.M.[2]
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Metagenomic and metatranscriptomics are providing tremendous insights into the diversity and the functionality contained within the communities of the human GI microbiota. A goal of the Human Microbiome Project is to access whether there is a "core human microbiome", an aspect of the microbial ecology that all healthy humans share. Yet, almost all studies to date are limited to clinical samples from industrialized nations. These samples are subject to bias resulting from medications, such as antibiotics, or introductions of microbes from the global catchment of food resources that may disrupt the natural state of human microbiome diversity.

The present study is unique because samples are drawn from communities with largely local diets, reducing such bias. This study presents human microbiomes inferred from fecal samples collected from remote areas of Peru. A traditional Matsés community from the Gálvez River, in the Loreto Region, and a traditional African American community from the coast of Ica-Peru are currently under investigation. As powerful as molecular-based methods are, it is essential to recover cultivars of representative taxa found within these communities. Physiological capabilities can then be attributed to individual taxa that contribute to this complex ecosystem. A benchmark for success is to recover organisms from the fecal samples transported from remote native communities in Peru. Organisms will be recovered from a series of broad based enrichments and from more selection media preparations to target taxa revealed from 454 pyrosequencing studies. Data will be presented on both molecular-based and cultivation based approaches providing insights into these remote communities and the structure of the microbial ecology of the GI microbiota. *The primary goal is to test the hypothesis that human gut microbes from remote geographic regions are unique in both taxa represented (phylotypes) and functional genomic potential.*

GUT MICROBIOTA IN OBESITY: PRELIMINARY RESULTS

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Departments of [1]Medical Microbiology;
[2]Health Promotion & Obesity Treatment;
[3]Pathophysiology, Medical University of Silesia, Katowice, Poland
[4]Department of Histology and Embryology Warsaw Medical University, Warsaw, Poland

Gut microbes are essential to metabolize food into energy, produce micronutrients and shape immune systems, they are also increasingly being linked to medical conditions including inflammatory bowel disease, diabetes, obesity and others. The aim of this study was to evaluate differences in influence of gut microflora on resting energy expenditure (REE) in patients with and without obesity. 50 patients with obesity (BMI ≥ 30 kg/m^2 - 39 females and 11 males) and 30 with normal body mass index (BMI 18,5-24,9 kg/m^2-24 females and 6 males) were included in this study. Patients with endocrin and infectious diseases were excluded. Body mass components were measured with Bodystat 1500 analyser, REE was calculated with indirect calorimetry. Presence of *Clostridium difficile* toxins A/B, *Clostridium perfringens* enterotoxin (TechLab, USA), *Helicobacter pylori* antigen (HpS, Meridian Bioscience, USA) and fecal lactoferrin (FLA) level (IBD-SCAN, TechLab, USA) were measured in each fecal sample, obtained from studied patients. Each fecal sample was appropriately diluted in pre-reduced PBS and plated on selective solid media for aerobic and anaerobic (Whitley A35, UK) quantitative cultures, respectively. Additionally, each sample was subjected to heat shock for 10 min. and cultured anaerobically. Isolated colonies were encountered, microorganisms were isolated and identified with use of ANC cards in Vitek 2 compact (bioMerieux, Marcy L'Etoile, France).

C. perfringens enterotoxin was not detected in evaluated samples. A and B toxins of *C. difficile* were demonstrated only in 2 (4.2%) samples obtained from obese patients. *H. pylori* antigen Ag was detected in fecal samples of 33.3% of obese patients and in 35.7% of controls. Significant qualitative and quantitative differences among isolated morphotypes of lactobacilli were not detected between both studied groups of patients.

REE (kcal/d) in patients with obesity was significantly higher compared with control group. Oxygen uptake and VCO_2 releasing were also significantly higher in group of obese patients. Number of colonies cultured from fecal samples of patients with obesity was higher compared with controls. Positive correlations were demonstrated between REE and total number of colonies from fecal samples (r=0.26, p=0.026) and negative correlations with percentage of *Firmicutes* (r=-0.24, p=0.04). Similarly, REE calculated in kcal/m^2/h positively correlated with total number of growing colonies (r=0,25, p=0,02), relations of *Bacteroides* to *Firmicutes* (r=0.26, p=0,02) and number of *Bacteroides* (r=0.24, p=0.03), but negatively with *Firmicutes* (r=-0.24, p=0.03). REE (kcal/kg/h) was reverse-proportional to percentage of *Firmicutes* (r=-0.48, p=0.008). FLA level was higher in group of obese patients compared with controls, however non-significantly. In our knowledge this is one the first studies evaluated influence of gut microflora on (REE) in patients with obesity. Further, more detailed studies directed to differences in virulence and genetic characteristics of isolated bacterial strains from patients with and without obesity are required to explain obtained results.

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1635 SESSION VI: WHAT'S HOT

SVI-1

WHAT'S HOT IN THE ANAEROBIC LITERATURE: BACTEROIDES

Lorber, B.*
Temple University School of Medicine, Philadelphia, PA USA

Review of important papers from the past year's literature that relate to Bacteroides and other medically important anaerobic gram-negative rods. These publications investigate changing antimicrobial susceptibility, the role of anaerobic gut flora in the development of the normal immune system (along with the maintenance of physical and mental health), and the potential role anaerobes play in the development of cancer.

WHAT'S HOT IN THE ANAEROBE LITERATURE: *CLOSTRIDIUM DIFFICILE* INFECTIONS

Nord, C.E.*
Department of Laboratory Medicine, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden

Clostridium difficile can cause antibiotic-associated diarrhea, colitis and pseudomembranous colitis, collectively known as *C. difficile* infections. It has been described in the literature since the late 1970s, and outbreaks continue to occur despite breakthroughs in laboratory and clinical diagnosis, effective treatments and infection control programs. During the last two years, more than 100 papers have been published in the field. The following aspects will be covered: diagnostic methods for laboratory detection of *C. difficile*, risk factors and recurrences in *C. difficile* infections, treatment options, and infection control of *C. difficile* infections.

800 SESSION VII: STATE OF THE ART LECTURE II

VAGINAL HEALTH: ECOLOGY AND DISEASE

Marrazzo, J.M.*
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The healthy vaginal environment favors reproductive health—in particular, delivery of healthy infants—and is dominated by H_2O_2-producing lactobacilli specific to the human vagina that maintain characteristically low pH (<4.7). Although the vaginal microbiome is dynamic, with daily sampling revealing marked changes in bacterial concentrations that can sometimes be related to sex, menses, or other external factors, sustained disruption in the ratio of key lactobacilli to commensal anaerobes can result in bacterial vaginosis (BV). BV involves loss of the "normal" hydrogen peroxide-producing lactobacilli and acquisition of complex bacterial communities that include many fastidious BV-associated bacteria (BVAB). BV is a common cause of vaginitis and increases women's risk of pelvic inflammatory disease, adverse pregnancy outcomes, and risk of STD/HIV acquisition. Recent evidence in populations at high risk for HIV acquisition suggests that BV increases affected women's risk of transmitting HIV to their male sex partners. The etiology of BV is unclear, though risks reflect a sexual component: sex without a condom, multiple partners, sex with women, and sex with an uncircumcised male partner. Treatment failure is common, and is facilitated by unprotected sex. Women whose male partners are uncircumcised are more likely to have BV, and uncircumcised men harbor anaerobes that have been strongly associated with BV, and that may be an independent cause of genital ulcer disease. Potential contributions to BV and BV persistence include (1) sexual partners as a reservoir for BVAB; (2) specific sexual practices, including male partners' condom use and inoculating anatomic site; (3) extravaginal reservoirs for BVAB, and (4) the composition of the vaginal microbiota involved in BV. While *Gardnerella vaginalis* plays a major role in BV, other BVAB in the Clostridiales Order are considerably more specific for BV, and may predict BV persistence when detected pre-treatment. BVAB colonization of men may serve as a reservoir for re-infection of women; specific sexual practices may favor vaginal colonization with certain BVAB that have been associated with persistence. This session will provide background on BV, and discuss recent developments in our understanding of the epidemiologic and microbiologic data that inform understanding of this complex dysbiosis.

945 SESSION VIII THE GENITAL MICROBIOTA

CLOSTRIDIUM VAGINAL INFECTIONS

Limbago, B.M.*
Centers for Disease Control and Prevention, Atlanta, GA USA

Review of the current literature related to clostridium vaginal infections.

SVIII-2

ANAEROBES IN PELVIC INFLAMMATORY DISEASE: AN UPDATE

Hillier, S.L.;*[1,2] Wiesenfeld, H.[1,2]
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[2]University of Pittsburgh, Department of Obstetrics, Gynecology, and Reproductive Sciences, Pittsburgh, PA USA

Pelvic inflammatory disease (PID) remains a leading cause of tubal factor infertility worldwide. Studies conducted from 1970-1980 suggested that most PID was caused by sexually transmitted pathogens including *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (CT). However, studies employing sample collection of the fallopian tubes of women with PID demonstrated that half of women having gonococcal salpingitis also had anaerobic bacteria present in the fallopian tube (Sweet, 1981). The prevailing theory was that STIs were the primary pathogens and that there was a secondary invasion of the upper genital tract by facultative and anaerobic bacteria colonizing the vagina. With increased screening and treated for STIs, the proportion of women presenting with symptoms and signs consistent with PID and having documented infection due to GC and CT have decreased. In an ongoing study of women presenting with acute PID symptoms in Pittsburgh, PA, only 16 (14%) have either GC or CT detected by NAAT, while 56 (49%) have facultative and/or anaerobic microorganisms recovered from an endometrial biopsy samples. *Gardnerella vaginalis* was the single most common isolate recovered from women with PID, being recovered from 24 (24%) of women without CT or GC, and from 6 (38%) of women with CT and GC. A total of 105 obligately anaerobic isolates were recovered from the endometrial samples, with anaerobes being detected in the endometria of 39% of women without CT or GC, and among 75% of those with STI pathogens. Anaerobic gram negative rods were recovered from the upper genital tract of 12 (11%) of women, anaerobic gram positive cocci from 13 (11%) of the women, while anaerobic gram positive rods were recovered from 23 (20%). The anaerobic gram positive rods included several novel organisms including *Atopobium vaginae*-like and two *Firmicutes* organisms which have not been previously cultivated. Novel anaerobic gram negative cocci included both *Megasphaera*-like type 1 and type 2. This ongoing study of women having acute symptoms of PID suggest that a lower proportion of women with PID have STI pathogens when comparing data from previous decades to the present. These data also suggest that a broad range of anaerobic microorganisms including some novel anaerobic gram positive rods and negative cocci which were thought to be noncultivable are being identified in the upper tract of women with PID.

1100 SESSION IX: STATE OF THE ART LECTURE III

SIX-1 Fidaxomicin & New Agents in the Treatment of *Clostridium difficile* Recurrences 30

*Louie, T.J.**

SIX-1

FIDAXOMICIN & NEW AGENTS IN THE TREATMENT OF *CLOSTRIDIUM DIFFICILE* RECURRENCES

Louie, T.J.*
University of Calgary, Calgary, AB Canada

Review of the latest research on the use of Fidaxomicin and other new agents in the treatment of recurrences of *Clostridium difficile*.

TIME *SESSION X: CLOSTRIDIUM DIFFICILE:* PATHOGENESIS, EPIDEMIOLOGY & THERAPY

SX-1

DEVELOPMENT OF A MURINE INFECTION MODEL TO STUDY *CLOSTRIDIUM DIFFICILE* DISEASE AND TRANSMISSION

Lawley, T.D.*
Bacterial Pathogenesis Laboratory, Wellcome Trust Sanger Institute, Hinxton, UK

Clostridium difficile rapidly emerged in the past decade and is now the leading cause of antibiotic-associated diarrhoea and a significant healthcare-associated pathogen in hospitals worldwide. The hamster infection model has served as a valuable surrogate to understand *C. difficile* disease, especially during the early stages of infection. To complement the hamster model, we developed a murine infection model that allows us to study host susceptibility and host-pathogen interactions as well as persistent infection and host-to-host transmission. This presentation will give an overview of the *C. difficile* mouse infection model and it's uses to study *C. difficile* biology.

COMMUNITY-ASSOCIATED *CLOSTRIDIUM DIFFICILE:* HOW REAL IS IT?

Lessa, F.*
Centers for Disease Control and Prevention, Atlanta, GA USA

In recent years, the epidemiology of *Clostridium difficile* infection CDI has changed dramatically, with increases in incidence and severity of cases being reported across the United States, Canada, and Europe In at least one U.S. region, *C difficile* has replaced methicillin-resistant *Staphylococcus aureus* (MRSA) as the most common cause of health care–associated infection. The recently changing epidemiology has also involved the emergence of CDI in populations previously thought to be at low risk, including severe cases among healthy peripartum women, and increasing reports in children and healthy people in the community with minimal or no recent exposure to healthcare settings. Recent studies have suggested that up to 30% of cases of CDI may be community-associated. The epidemiology and risk factors for community-associated CDI have not been well explored.

The objectives of this presentation will be to: (1) review available data on community-associated *C. difficile* epidemiology, and (2) discuss current prevention strategies for CDI outside acute care settings

SX-3

CLOSTRIDIUM DIFFICILE IN FOOD: SUPERMARKET REALITY OR LABORATORY CONTAMINANT?

Marsh, J.*
University of Pittsburgh School of Medicine, Pittsburgh, PA USA

The prevalence of *Clostridium difficile* in food animals and the reported increase in community-associated *C. difficile* infections has led to speculation of transmission to humans through retail foods. Multiple studies report the isolation of *C. difficile* from retail foods including fresh meats, ready-to-eat- meats, and vegetables with prevalence estimates ranging from 0 – 42%. Evidence suggesting transmission from farm to table comes from molecular typing methods. PCR ribotype 078, commonly found in food animals, has recently emerged in the Netherlands causing severe disease in otherwise healthy individuals. PCR ribotype 027, the causative agent of multiple world-wide epidemics has been isolated from retail meats in Canada and Arizona. Reports of *C. difficile* in the food supply are of public health concern. *C. difficile* is ubiquitous in nature and contamination of retail foods can occur at multiple levels – from the farm soil, to the meat processing facility, to the supermarket employees and restaurant chefs, to the cramped quarters of the laboratory anaerobic chamber. Strict measures to control *C. difficile* contamination in the laboratory are necessary to ensure the validity of food sampling results. In addition, molecular genotyping methods with sufficient discriminatory power must be used to accurately attribute potential *C. difficile* transmission events. Currently, multi-locus variable number tandem repeat analysis (MLVA) is the most discriminatory method for *C. difficile* typing. This method has proven utility for tracking *C. difficile* transmission in the hospital and is routinely used for infection control purposes at our hospital. More recently, MLVA has been applied to investigations of *C. difficile* in food animals and food and the potential association with human disease. Recent studies suggest that ribotype 078 isolates of porcine and human origin are highly related by MLVA. However, MLVA has limited discriminatory power for PCR ribotype 078 and conclusions regarding genetic associations of human and animal isolates must be made cautiously. Investigation of ribotype 027 isolates from retail meat identified several clusters of food, animal and human isolates that were highly related or indistinguishable by MLVA. Widespread dissemination of a clonal *C. difficile* population may explain these results. However, identity by MLVA is rarely observed even in serial isolates from the same patient. An alternative explanation is laboratory contamination during the labor intensive processing of multiple food samples. Further investigations of food are necessary to establish that transmission of *C. difficile* from the food supply to humans occurs. Rational food sampling plans paired with rigorously controlled laboratory procedures and the collaboration of veterinary, food industry, and molecular epidemiologic scientists are required to address this important question.

CLOSTRIDIUM DIFFICILE IS AN AGENT OF FOODBORNE DISEASE

Songer, J.G.*
Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA USA

Clostridium difficile is well known as a human pathogen, causing antibiotic-associated diarrhea, pseudomembranous colitis, and in its most severe form, paralytic ileus, bowel perforation, peritonitis, and death. Infections are often hospital-acquired, with symptoms appearing after colonization of the bowel by *C. difficile* while the patient is hospitalized, followed by alteration of the bowel microbiome by treatment of the patient with antimicrobials. However, there is general agreement that community-associated disease is increasing in frequency, as well as general agreement that we do not know the source of the infecting organism.

Given the finding of identical strains of *C. difficile* in humans and in food animals, interest arose in the possibility of an animal source for human infections. This manifested in study of foods, with cultural examination of retail meats and other potential sources of the organism. Scott Weese's group was apparently the first to document the presence of *C. difficile* in retail meats, and they were soon joined by many others. Ground beef, ground pork, pork sausage, pork liver paté, and various other uncooked or ready-to-eat items were examined by working groups in the US, Canada, Europe, and elsewhere. Even if, as hypothesized by others, some isolates are the result of contamination in the laboratory, there is no question that retail meats can be a rich source of *C. difficile*.

The paradigm for clostridial foodborne disease is infection by enterotoxigenic *C. perfringens* type A. Spores contaminating meats (typically) germinate and multiply as a result of poor food handling practices. Large numbers of vegetative cells are ingested, and conditions unfavorable for this form of the organism induce sporulation, which is accompanied by enterotoxin production. In the case of *C. difficile*, some of the events could be identical. Cooking may kill some, but certainly not all, spores in meats, and temperature abuse after cooking is as likely to have the same effect on *C. difficile* as on enterotoxigenic *C. perfringens*.

Nonetheless, it may not be possible to assemble a cohort of individuals developing *C. difficile* infection (CDI) and assign the origin of the disease to a foodborne source; alignment of a multitude of planets is required for disease to result, even if colonization has been accomplished. Research is continuing.

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1500 SESSION XI: CLOSTRIDIUM DIFFICILE: **ORAL PRESENTATIONS**

SXI-1

THE CHANGING FACES OF *CLOSTRIDIUM DIFFICILE*: A PERSONAL REFLECTION OF THE PAST 34 YEARS

Poxton, I.R.*
Medical Microbiology, University of Edinburgh College of Medicine and Veterinary Medicine, Edinburgh, UK

Sometime late in 1978, my then boss, Gerry Collee, handed me a folder with "*Clostridium difficile* (diffikilé)" written on it. Inside were a few recent and now classic papers by Bartlett, George, Larson and co. Gerry suggested that I begin to work on what he felt might be an interesting problem. It was the beginning of a continuing adventure which has resulted in at least 50 publications from my group.

Over the years, I like to think we have made several important contributions to the field. Beginning in 1982, we showed that *C. difficile* was a common cause of community acquired infection! During the next few years, we did extensive structural studies on the bacterium. This culminated in 1984 with a fingerprinting study (by immunoblotting surface antigens), done with Swedish strains supplied by Carl-Erik Nord, which probably was the first to demonstrate conclusively that it was really an infectious agent. This was later reinforced with strains sent from Amsterdam by Ed Kuijper. Later in the 80s, in a study of recurrent disease, we showed that ca. 50% of recurrences were due to infection with a different strain. During my term as chair of the European Study Group for *C. difficile,* we began to define the status of *C. difficile* infection (CDI) in Europe and develop guidance for diagnosis and treatment. Recently, we utilised our extensive culture collection, with isolates from the 1970s to the present, to observe how epidemiology has been driven largely by antibiotic usage. We have now come full circle: in the early years CDI was caused by a multitude of different strains. Then in period beginning in the 1990s, characterised by often large outbreaks and poor infection control, disease was caused by just a few endemic strains highlighted by the 027/NAP1/BI pandemic. Now in a much improved current situation, at least locally in Scotland, we are seeing again that the majority of cases (largely sporadic) are caused by multiple types.

Our recent work is equally diverse ranging from molecular studies on toxin and spore production, to immune responses, to novel observations on CDI in children, to what is the best way of decontaminating *C. difficile* spores from your anaerobe laboratory.

COLONIZATION RESISTANCE: INTERACTIONS BETWEEN *CLOSTRIDIUM DIFFICILE* AND THE INDIGENOUS GUT MICROBIOTA

Young, V.B.*
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Department of Microbiology and Immunology,
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Current theories regarding the pathogenesis of *Clostridium difficile* infection (CDI) posit that antibiotics disrupt the indigenous gut microbiota and eliminate colonization resistance against the organism. We have been studying the interaction between *C. difficile* and the gut microbiota in patients with CDI and in experimentally infected mice. Through the use of culture-independent methods to profile the community structure of the gut microbiome, we have shown that different community structures have differential ability to prevent colonization with *C. difficile* and influence subsequent pathogenesis. However, we still do not understand the specific mechanisms by which the microbiome can interfere with the ability of *C. difficile* to colonize and grow within the intestinal ecosystem. To gain insight into some of these mechanisms, we have used advanced culture techniques to isolate specific members of the gut microbiota that are associated with colonization resistance against *C. difficile*. In particular, we have focused on members of the family Lachnospiraceae, which includes a number of organisms that produce the short chain fatty acid butyrate. Butyrate has been shown to be essential for maintaining normal physiology of the gut epithelium and may have specific activity against *C. difficile*. We have demonstrated that colonizing germ-free mice with specific members of the Lachnospiraceae can interfere with colonization and the development of colitis in animals experimentally challenged with *C. difficile*. Additionally, to identify other metabolic products produced by the indigenous gut microbiota, we are using a broad metabolomic approach to determine how antibiotic administration disrupts the normal intestinal milieu, thus eliminating colonization resistance and opening the door to the development of CDI. We hope that a better understanding of the mechanisms by which the indigenous microbiota prevents the ingrowth of pathogens will open the door to novel measures to prevent and treat CDI.

SXI-3

EPIDEMIOLOGY OF COMMUNITY-ASSOCIATED *CLOSTRIDIUM DIFFICILE* INFECTION (CA-CDI), EMERGING INFECTIONS PROGRAM, 2009–2011

Chitnis, A.;*[1] Holzbauer, S.;[1,2] Belflower, R.;[1] Winston, L.;[3] Kast, K.;[4] Lyons, C.;[5] Farley, M.;[6] Perlmutter, R.;[7] Dumyati, G.;[8] Beldavs, Z.;[9] Dunn, J.;[10] Gould, L.H.;[1] McDonald, C.;[1] Lessa, F.[1]
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[7]Maryland Department of Health and Mental Hygiene, Baltimore, MD USA
[8]University of Rochester, Rochester, NY USA
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[10]Tennessee Department of Health, Nashville, TN USA

Purpose: To describe the epidemiology, ambulatory healthcare exposures, and community-based sources of *C. difficile* among CA-CDI cases from 1/1/2009 to 5/31/2011.
Methods and Results: Prospective, active population-based CDI surveillance. A CA-CDI case was defined as a positive *C. difficile* toxin assay on a stool specimen from a person with diarrhea who had neither a prior positive assay within 8 weeks nor an overnight healthcare facility stay within 12 weeks before stool collection. Data on demographics, ambulatory healthcare exposures, antimicrobials, household members, and food were collected through medical record review and telephone interviews. Ambulatory healthcare exposures included surgery or a procedure, dialysis, care at an emergency/urgent care facility, or a job requiring direct patient-contact 12 weeks before stool collection. Cases with and without prior ambulatory exposures were compared using chi-square tests. Variables with *P*-value <0.20 were eligible for inclusion in a logistic regression model.
Of 989 cases, 64% received antimicrobials and 41% had ambulatory exposures. Mean age was 48 years, 67% were female, and 86% were white. Cases without ambulatory exposures were more likely (P <0.05) to have no reported medical conditions, an infant aged <1 year in the household, and a household member with CDI, and less likely to have received antimicrobials than cases with ambulatory exposures. Adjusting for medical conditions and household member with CDI, an infant <1 year in the household was associated with CA-CDI without prior ambulatory exposure (aOR=2.06; P= 0.05).
Conclusion: Prevention of CA-CDI should focus on reducing antimicrobial use and possible *C. difficile* transmission in ambulatory settings. New measures to prevent *C. difficile* transmission in the home may be warranted.

THE IMPORTANCE OF TOXIN A IN VIRULENCE OF AN EPIDEMIC *CLOSTRIDIUM DIFFICILE* STRAIN

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Clostridia Research Group, School of Molecular Medical Sciences, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK

Clostridium difficile infection (CDI) is the main cause of healthcare acquired diarrhoea in the developed world, where it imposes a significant financial burden and leads to higher mortality rates than MRSA. *C. difficile* produces two main virulence factors—toxin A and toxin B. However, their respective roles in disease remain controversial. Notably, whilst two recent studies using isogenic toxin mutants of *C. difficile* both showed the importance of toxin B: one study demonstrated that toxin A alone could not cause disease in hamsters (Lyras *et. al.*, 2009) whereas the other showed that an equivalent mutant in the same animal model was virulent (Kuehne *et. al.*, 2010). The mutants used in both studies were generated in erythromycin sensitive derivatives of strains 630, which had been isolated independently after repeated serial passage. Therefore, the emergence of ancillary mutations that impact on virulence cannot be discounted. This is currently being investigated through genome re-sequencing and phenotypic experiments. In the meantime we have repeated the study in another strain, R20291 which is a representative of the PCR-Ribotype 027 clade (027/NAP1/B1) often associated with increased disease incidence and severity. This epidemic strain does not only produce toxin A and B, but in addition a binary toxin, called *Clostridium difficile* Transferase (CDT). Through the creation of stable, isogenic toxin mutants in R20291, using ClosTron technology, we demonstrate, like in our first study (Kuehne *et. al.*, 2010), that each of the two main toxins alone can cause fulminant disease in the hamster model of infection. Furthermore we observed a perhaps synergistic effect of CDT with toxin A and B. Our findings re-establish the importance of toxin A and B in CDI and emphasize a need to consider both when developing effective countermeasures.

SXI-5

PHENOTYPIC CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* CLINICAL ISOLATES

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Specific ribotypes of *Clostridium difficile* (*Cd*), associated with epidemic spread and increased disease severity, have been characterized as "hypervirulent". This "hypervirulence" has been associated with enhanced sporulation and toxin production. However, the extent to which ribotype is associated with specific virulence phenotypes *in vitro* or predicts clinical disease severity in patients remains unknown. We sought to determine whether there is a correlation between multiple *Cd* phenotypic characteristics and either strain ribotype or clinical disease severity. More than 100 *Cd* strains, representing 13 different ribotypes and isolated from symptomatic patients, were analyzed. Detailed clinical information was available for 89 of the cases, which were diagnosed at the University of Michigan. Severe infection was defined according to 2007 CDC definitions. All strains were tested for germination efficiency, sporulation, spore viability, and growth rate. The tested isolates exhibited a wide range of phenotypes. All were capable of full germination within five minutes of exposure to bile salts. In contrast, the ability of isolates to sporulate was variable, with greater than a 100 fold difference observed across these strains. Even greater differences were observed in spore viability, with 1-83% of observed spores being capable of outgrowth. Although significant differences were observed in each of these assays, these results could not be correlated to a strain ribotype or clinical disease severity. Interestingly, a significant correlation between *Cd* growth rate and clinical disease severity was observed. Strains associated with severe disease exhibited faster growth than those associated with non-severe infection ($p > 0.0001$). We speculate that differences in growth rate will correlate with differences in toxin production (currently under assessment). We have newly identified a link between bacterial growth rate *in vitro* and clinical disease severity that is independent of ribotype. This study represents one of the first reports correlating a specific phenotype of *Cd* isolates and disease severity.

SEVERE *CLOSTRIDIUM DIFFICILE* ASSOCIATED DIARRHEA—IMPACT OF HIGH DOSE VANCOMYCIN THERAPY

Schmitt, D.V.;[1] Claros, M.C.;*[2] Tauchnitz, R.;[3] Mohr, F.W.;[1] Rodloff, A.C.[4]
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[4]Institute of Medical Microbiology, University of Leipzig, Leipzig, Germany

Severe *Clostridium difficile* (CD) associated diarrhea (CDAD) is an emerging infectious disease often associated with severely ill, hospitalized patients. Although metronidazole has been used to treat these patients, it often leads to treatment failure. Oral vancomycin (125 mg, 4x / day for 10 days) is the currently recommended therapy for CDAD. Unlike metronidazole, which is resorbed by gastrointestinal enterococci, vancomycin is not resorbed in the gut. In theory, therefore, vancomycin therapy should be successful in treating CDAD. We have been monitoring CDAD cases in the cardiac surgery intensive care unit (ICU) at the Heart Center University of Leipzig for two years. During that time, we noted that the number of CDAD cases increased dramatically. The standard vancomycin therapy led to relapses, i.e., recurrence of symptoms and any positive CD assay after cessation of therapy, in our severely ill ICU patients. Furthermore, several CDAD patients required surgical intervention due to severe toxic forms of CDAD. In order to address these problems, we modified the oral vancomycin therapy regimen and introduced additional infection control measures. At that time, the ICU contained 80 beds, and served 3,838 patients with 34,699 patient days. Stools of patients who showed clinical symptoms suggesting CDAD were submitted for CD culture and toxin A/B ELISA. Symptomatic patients with a positive culture and/or ELISA result were kept in isolation and treated with a high dose of oral vancomycin (500 mg, 4x / day for 14 days). Thus, we found that the high dose vancomycin therapy was effective in reducing both the number of relapses and the necessity for surgical intervention in patients with CDAD.

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***1630 SESSION XII:* UNKNOWN CASES**

Gonzalo Ballon-Landa, M.D.
Ellen Jo Baron, Ph.D.
Ellie J.C. Goldstein, M.D.

Clinical cases involving anaerobic bacteria will be presented in a Grand Rounds format. Panelists and audience members will have the opportunity to respond and ask questions to determine the etiology and suggest interventions.

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815 SESSION XIII: STATE OF THE ART LECTURE IV

SXIII-1

ANAEROBIC DIAGNOSTIC MICROBIOLOGY: *CLOSTRIDIUM DIFFICILE* AND BEYOND

Citron, D.M.*
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Isolation and identification of anaerobic bacteria remains problematic for many clinical laboratories. Some commercial culture media may not be optimal for more fastidious strains, resulting in the perception that anaerobes are difficult to grow and leading some labs to abandon the effort. Good culture media should be fresh and include selective and differential agars that can provide important information rapidly. These include BBE agar that grows the *B. fragilis* group, some fusobacteria, and *Bilophila,* all of which produce distinctive colonies. LKV agar grows *Prevotella* spp. with enhanced pigment production and also *Fusobacterium necrophorum*. PEA medium inhibits enteric bacteria and grows most anaerobes including the anaerobic cocci. Anaerobes should remain in an anaerobic atmosphere and not be exposed to oxygen especially during the log phase of growth. Identification using the rapid identification kits provides genus level of identification for 70-90% of clinical isolates, but species level identification is less accurate, due in part to the rapidly changing taxonomy that is not reflected in the data bases, but also because of overlapping or difficult to interpret reactions for some organisms. Because of the need for pure cultures and a heavy inoculum for inoculation of these kits, identification is delayed by several days after receipt of the specimen, and the relevance of the identification decreases. Clearly, a better method is needed. The MALDI-TOF mass spec requires only one colony for identification, and the data bases are currently under development with more anaerobic species being included, making this method promising for the future. Some laboratories have access to 16S rRNA gene sequencing, which has become the gold standard for identification of most bacterial species. Other genes may also be sequenced for typing or for species with similar 16S sequences. Toxigenic culture for diagnosis of *C. difficile* infection is becoming increasingly popular. Several modifications of the original CCFA medium have enhanced rapid recovery of this organism from stool, and toxin may be tested directly from 48h colonies using an ELISA method. Given the right methods, anaerobic cultures can be done as easily as aerobic cultures.

IS MALDI-TOF MS SUPERIOR TO COMMERCIALLY AVAILABLE IDENTIFICATION KITS FOR IDENTIFICATION OF ANAEROBIC BACTERIA IN ROUTINE LABORATORIES?

Nagy, E.;*[1] Becker, S.; [2] Kostrzewa, M.;[2] Urbán, E.[1] on behalf of ESGAI
[1]Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary
[2]Bruker Daltonik GmbH, Bremen, Germany

Clinically relevant anaerobic bacteria needs relatively long time for isolation in pure culture and their identification is hindered by the inactivity of many species in biochemical tests or by the difficulties to reach enough inokulum for identification in real time. MALDI-TOF MS is a promising method to overcome many problems concerning anaerobic bacteria, if the data base is broad enough to cover most of clinically relevant anaerobic species. During a 4-year period, a collaborative study was carried out between the National Anaerobe Reference Laboratory for Hungary and Bruker Daltonics, Bremen, Germany to challenge the available data base and to improve it by including new species and more clinical isolates. Comparison with data obtained by the use of commercially available identification kits was carried out and 16S rRNA gene sequencing was used for strains with low log(score) or discrepant results.

Between 2008 and 2011, clinically relevant non-duplicate anaerobic isolates (473) were identified—if possible—by different traditional methods, such as growth on different selective media, presumptive identification according to the Wadsworth Manual and rapid ID 32A ATB and API20 ANA (BioMerieux) kits in the Anaerobic Reference Laboratory of Hungary. Incubation was carried out in an anaerobic chamber (Bactron, USA). Part of these strains were a subset of an Europe-wide antibiotic resistance study for *Bacteroides* strains. Immediately after isolation, an ethanol extraction was carried out on isolated colonies and the stabilized samples were sent to the Bruker Laboratory in Bremen, Germany, where the identification was done by using the standard protocol of the MALDI-TOF MS (Microflex) and the spectra were imported into the Biotyper software (version 2.0).

Out of 277 *Bacteroides* strains, 270 was correctly identified by MALDI-TOF MS with a log(score) >2. The 7 isolates which gave inconclusive identification were sequenced and added to the data base (*P. distasoinis, P. goldsteinii, B. eggerthii*, and *B. intestinalis*). Out of the further 196 non-duplicate anaerobic clinical isolates from different genera (including *Bacteroides*, *Prevotella*, *Fusobacterium, Clostridium*, *Peptostreptococcus*, *Finegoldia, Propionibacterium* and some unidentified Gram-negative and Gram-positive anaerobic bacteria) MALDI-TOF MS identified 166 (84.6%) strains at a species level and 184 (93.8%) at a genus level. After 16S rRNA gene sequencing, it turned out that for 10 isolates the species was not included into the database. In cases of discrepant phenotypic identification, 16S rRNA gene sequencing supported the MALDI-TOF identification in 97% of the cases. Even species, which are difficult to be distinguished by commercially available identification kits (such as *B. fragilis* and *B. capillosus*) were correctly identified. By including newly sequenced anaerobic species from our strain collection into the database, the "missed" results could be minimised. For *B. fragilis* (division I and II) and *P. acnes* (types IA, IB, II and III) the MALDI TOF MS method was also used successfully for typing.

As a conclusion, MALDI-TOF MS seems to be a very promising identification method, especially in the case of anaerobic bacteria, which need a special culture condition, a longer incubation time to get proper growth, and are biochemically often inactive.

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1000 SESSION SXIV: OTHER CLOSTRIDIAL INFECTIONS

SXIV-1

BOTULISM: NEWER ASPECTS

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Botulism is an acute flaccid paralytic disease of humans and animals that results from the action of botulinum neurotoxin at peripheral cholinergic synapses, principally the neuromuscular junction. Five forms of human botulism are known: infant (which rarely occurs in adults), foodborne, wound, inhalational, and physician-associated. For more than 20 years infant botulism has been the most common form of human botulism in the United States; it results from swallowed spores of *Clostridium botulinum* (and rarely neurotoxigenic *C. butyricum* and *C. baratii*) that activate and produce botulinum toxin in the large intestine. Botulinum toxin is the most poisonous substance known and a "maximum threat" potential bioweapon via deliberate food contamination or aerosolization. The astute clinician remains the essential element in rapid recognition of naturally occurring or bioterrorism botulism.

Diagnostic capability, treatment, and prevention of botulism have all advanced in the past decade. The mouse protection assay that demonstrates biologically active botulinum toxin remains the cornerstone of diagnosis but is slow; a screening DIG-ELISA and real-time PCR are being used by the Laboratory Response Network. Some research laboratories have developed sensitive mass spectroscopy, FRET, ALISSA, and physiological assays. A recently approved neuronal cell-culture assay is facilitating production of therapeutic botulinum toxin.

The seven botulinum toxin types A-G are distinguished diagnostically and therapeutically by the inability of a polyclonal antitoxin raised against one toxin type to neutralize any of the other six toxin types in the mouse protection assay. Since March 2010, CDC has provided an investigational equine $F(ab')_2$ Heptavalent Botulism Antitoxin to adult patients, while licensed Human Botulism Immune Globulin for infant botulism patients has been available since October 2003. A human-compatible monoclonal antibody-based antitoxin is in a Phase I clinical trial. Differences in toxin neutralization by monoclonal antibody combinations have resulted in identification of subtypes of toxins A, B, E and F.

The botulinum toxin gene, and its accessory genes, constitute the "toxin gene cluster," a mobile genetic element that may reside in the chromosome, a plasmid or a bacteriophage. Recent molecular characterization of *C. botulinum*, when combined with infant botulism epidemiological information, has provided new insights into the genetics and global geography of this diverse species that remains medically important despite infrequent case occurrence.

CLOSTRIDIUM SPP.: MECHANISMS OF DISEASE

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Clostridia represent a diverse group of spore-forming gram positive anaerobes that include several pathogenic species. In general, disease caused by clostridia is a result of intoxication of the infected host. Thus, clostridial toxins have been targeted for diagnostic, therapeutic, and preventive strategies against infection. Studying the mechanisms of action of clostridial targets has not only shed light on the pathogenesis of infection but has provided important new insights into cell biology and immunology. A primary purpose of this talk is to provide state-of-the-art review on the mechanisms of disease caused by clostridial intoxication. *C. sordellii*, *C. novyi*, and *C. tetani* will be among those bacteria highlighted in this lecture, as *C. botulinum*, *C. difficile*, and *C. perfringens* are being discussed in other lectures and abstracts.

Anaerobe 2012

1110 *SESSION XV* ORAL PRESENTATIONS: *CLOSTRIDIUM SPP.*

SXV-1

RECENT PROGRESS IN UNDERSTANDING THE ACTION OF *CLOSTRIDIUM PERFRINGENS* ENTEROTOXIN

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Clostridium perfringens enterotoxin (CPE) causes the gastrointestinal symptoms of *C. perfringens* type A food poisoning, which is the second most common bacterial foodborne illness in the USA, where an estimated 1 million cases/year occur. CPE is also important when CPE-positive *C. perfringens* cause ~5-15% of all cases of antibiotic-associated diarrhea. There is also current interest in employing CPE, or CPE fragments, for cancer therapy and to enhance drug absorption. Recent structural biology studies revealed that CPE belongs to the aerolysin pore-forming toxin family. The action of CPE begins with binding of the toxin to certain members of the claudin family of tight junction proteins. Extensive site-directed mutagenesis studies are now mapping those CPE: claudin interactions and results have indicated that the C-terminal portion of CPE interacting with the second extracellular loop of claudin receptors. The possible use of soluble claudin receptors as potential therapeutics to block CPE action is now being explored with cell cultures and animal models. After binding to a claudin receptor, CPE oligomerizes into a hexameric prepore on the membrane surface. The CPE prepore then inserts into the membrane to form an active pore. Ongoing site-directed mutagenesis studies suggest that a region of alternating hydrophobic/hydrophilic amino acids functions as a beta hairpin during toxin insertion and pore formation. These structure/function studies may improve the use of CPE for therapeutic purposes, as well as lead to new therapeutic approaches to block CPE-mediated disease.

EVIDENCE THAT THE AGR QUORUM SENSING SYSTEM IS A REGULATOR OF *CLOSTRIDIUM PERFRINGENS* TOXIN PRODUCTION AND VIRULENCE

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Clostridium perfringens, an important pathogen of humans and domestic animals, causes wound infections, e.g. gas gangrene, as well as intestinal diseases involving the intestines, e.g. food poisoning, necrotic enteritis and enterotoxemia. *C. perfringens* virulence is largely dependent upon prolific toxin-production, with this bacterium capable of producing at least 17 different toxins. The *agr* system is a well-characterized regulator, in a quorum-sensing (QS) manner, of toxin genes in *Staphylococcus aureus*. *C. perfringens* epsilon toxin (ETX) is considered the third most potent of all clostridial toxins and thus has been classified by the CDC as a class B select toxin. ETX is also a major virulence factor in several important natural veterinary enterotoxemias caused by type B and D strains. To date there is limited information available regarding the regulation of ETX and/or CPB production by type B, C or D strains. In order to understand toxin production regulation, *agrB* null mutants were generated by Targetron insertional mutagenesis technology in *C. perfringens* type B, C and D strains. We found that Type C or D *agrB* mutants exhibits strongly reduced CPB or ETX production compared with their wild-type parents. Interestingly, in type B strains, the Agr QS system can regulate CPB production, but it does not regulate ETX production *in vitro*. Initial animal testing has shown that the *agrB* mutant is attenuated for causing hemorrhagic necrotic enteritis in rabbits and fatal enterotoxemia in mice. Mouse lethality experiments demonstrated that the *agrB* mutant also exhibits less lethality compared with wild-type. The findings support the Agr-like QS system's global importance for regulating *C. perfringens* toxin production and pathogenicity.

SXV-3

HIGHLY SENSITIVE CELL MODEL FOR BOTULINUM NEUROTOXIN DETECTION USING HUMAN NEURONS FROM INDUCED PLURIPOTENT STEM CELLS

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Currently, several neuronal cell models for BoNT detection and mechanistic studies exist, but none examine toxin function with species-specific relevance while exhibiting high sensitivity. The most sensitive cell models are mouse or rat primary cells and neurons derived from mouse embryonic stem cells, which both require significant technical expertise for cell preparation. In this study, human neurons derived from induced pluripotent stem (hiPS) cells were examined for their potential as a novel *in vitro* cell model for BoNT detection and study of cell biology.

The hiPS neurons expressed all known receptors and substrates required for BoNT intoxication. These neurons were highly sensitive for detection of BoNT/A, B, C, and E, with EC_{50} values of about 0.3, 16, 0.4, and 1.8 mouse LD_{50} Units, respectively. Compared to primary rat spinal cord (RSC) cells, the human iPS cell-derived neurons had a tighter dose response curve, similar or lower EC_{50} values, and achieved full SNARE target protein cleavage with all BoNT serotypes tested. BoNT/A appeared to enter the hiPS cell-derived neurons faster than RSC cells in an activity-dependent assay. Neutralizing antibodies were detected with equal sensitivity to the RSC assay. In conclusion, neurons derived from hiPS cells provide an easy to use, highly sensitive, reproducible, and human *in vitro* cell model for BoNT activity and neutralizing antibody detection, and for continuing basic research.

PHENOTYPE MICROARRAYS AND CYTOTOXICITY-BASED CLOSTRIDIAL TOXIN ASSAY

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To develop a cytotoxicity-based clostridial toxin assay that is reliable, efficient, and quantitative and allows determination of culture conditions that induce or repress toxin production.
Methods: *C. difficile* strains ATCC 9689 and ATCC 43255 (API 10463), *C. perfringens* (ATCC 13124), and *C. sordellii* (ATCC 7914) were inoculated into Phenotype MicroArray (PM) panels (96-well format). After 72 hr of anaerobic culture, the well supernatants were harvested and a 5ul aliquot with or without dilution was transferred into a 96-well tissue culture microplate seeded with CHO-k1 cells. Cell morphologies were recorded after 18-20 hours of exposure to the toxin-containing supernatants. Cell viability was then determined with a redox dye and kinetic data was automatically collected on an OmniLog instrument for 3 hr. The dye reduction rate calculated using OmniLog analysis software was compared against standard toxin titrations to determine the concentration of toxin. A neutralization assay was also performed to demonstrate the specificity of the analytes.
Results: Purified toxin B of *C. difficile* showed a dose-dependent cytotoxicity on CHO-k1 cells, which is inversely correlated with dye reduction rate by the cells. Using CHO-k1, the measurable range of toxin B was about 0.1-1000 ng/ml. The cytotoxicity of the pure toxin B or the toxin B-containing supernatants of both *C. difficile* strains was neutralized by antitoxin B antibodies. Toxin production levels under different culture conditions are variable and can be strain-dependent. API 10463 produced > 1000 fold more toxin B than ATCC 9689 under certain culture conditions. Arginine dipeptides gave high levels of toxin production for *C. difficile* and *C. perfringens*, but not for *C. sordellii*. D-Glucose and D-trehalose strongly repressed toxin production by *C. perfringens*, but D-trehalose did not repress toxin production by *C. difficile* and *C. sordellii*.
Conclusions: The combination of PM technology with cytotoxicity assay provides a powerful tool to reliably, quantitatively, effectively, and efficiently measure cytotoxins and their regulation under a large number of culture conditions for *C. difficile*, *C. perfringens*, and *C. sordellii*.

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1315 SESSION XVI: PROBIOTICS: MECHANISMS & HEALTH BENEFITS

SXVI-1

PROBIOTICS IN VAGINAL INFECTION PREVENTION

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A review of research related to the effects of probiotics on vaginal infection prevention.

ANTIBIOTIC-ASSOCIATED DIARRHEA: ARE PROBIOTICS THE ANSWER?

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The role of probiotics, if any, in the prevention or treatment of AAD remains controversial. A striking feature of many reviews of the subject is the clear prejudice of the authors in favour of, or against the role of probiotics, regardless of the evidence under discussion. In addition, there have been a substantial number of careful meta-analyses and systematic reviews, probably more than the number of well designed trials. Given the heterogeneity of the probiotic preparations used, involving bacteria and fungi of several different genera, a huge variation in the doses used and often a lack of clarity over which particular strain has been used, it is remarkable that any effect is still discernible in a meta-analysis, but it does appear that the use of probiotics is associated with a reduction in AAD. In the UK, the incidence of severe AAD associated with *C. difficile* infections continues to decline, making large trials difficult to carry out, nevertheless only such large multi-centre trials, involving at least 500 subjects and using well characterised and carefully controlled probiotics, are likely to have sufficient power to decisively resolve the issue.

SXVI-3

PROBIOTIC MECHANISMS OF ACTION—ONE BUG'S STORY

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Lactobacillus rhamnosus GG (LGG) is one of the best-studied probiotic bacteria in clinical trials for treating and/or preventing intestinal disorders, such as diarrhea, necrotizing enterocolitis, and inflammatory bowel diseases (IBD). However, the clinical application of LGG and other probiotics has been limited by a paucity of mechanistic information. Our laboratory has used LGG as a model organism to dissect mechanisms of probiotic action. We have purified and cloned two soluble proteins (p40 and p75) from LGG that inhibit epithelial cell apoptosis via an epidermal growth factor receptor/phosphatidylinositol 3-kinase/Akt-dependent mechanism. We have extended these studies to *in vivo* mouse models of colitis. Our findings show that activation of EGFR by p40 is required for inhibition of cytokine-induced apoptosis and disruption of barrier integrity in intestinal epithelial cells *in vitro* and *ex vivo.* Furthermore, we developed a colon-specific protein delivery system using pectin/zein-based hydrogel beads, and show that p40 stimulates EGFR and Akt activation in colon epithelial cells of wild-type (wt), but not EGFR kinase defective ($EGFR^{wa2}$) mice or dominant negative ($EGFR^{wa5}$), indicating that p40 is biologically active at the colon epithelial cell *in vivo*. Treatment of mice with p40 prevents and treats in three different mouse models of colitis in an EGFR-dependent manner. Therefore, we conclude that p40 protects the intestinal epithelium *in vivo* by reducing epithelial cell apoptosis and preserving intestinal barrier integrity, in an EGFR-dependent manner. These studies define a previously unrecognized mechanism of action via probiotic-derived soluble proteins in modulating intestinal homeostasis and restricting inflammation, while providing evidence to support a novel therapeutic approach for treating intestinal inflammatory disorders.

1445 *SESSION XVII:* ORAL PRESENTATIONS

SXVII-1

GEOGRAPHIC SIGNATURES OF ANAEROBES TO STUDY HUMAN-MICROBE CO-MIGRATION AND CO-EVOLUTION

Horz, H.P.;[1] Stoneking, M.;[2] Li, J.;[2] Kessler, O.;[1] Sonanini, A.;[1] Schilling, H.;[1] Conrads, G.*[1]
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Since 100,000 years, the human microbiome has co-migrated and co-evolved with us. As a matter of fact, we share life and history with our microbial communities. For evolutionary studies, the resolution of human DNA itself (Single Nucleotide Polymorphisms, SNPs) is too low and limiting to answer all questions. In contrast, the genetic resolution of our residential bacteria is much higher and might thus help answer anthropological questions.
Purpose: Here we test the hypothesis that the genetic variability of distinct anaerobic bacterial species in the oral ecosystem may have the potential to serve as a chronometer of human evolution.
Methods: To this end saliva samples from ten volunteers, each from 12 areas world-wide, representing diverse ethnic groups, have been the initial focus of this study. Variations in the 16S-23S rDNA internal transcribed spacer region of *Fusobacterium nucleatum,* as well as the *gdh*, (encoding for the glucose-dehydrogenase) and the *gtf* (encoding for the glucosyl-transferase) of Mitis-Oralis streptococci (all of which are typical pioneers of biofilm formation), have been analyzed by culture-independent methods (direct PCR amplification, cloning and sequencing).
Results: We observed a high intra- and inter-individual clonal diversity for all species analyzed. Phylogenetic tree reconstruction revealed several clusters shared between two or more countries but also country-specific lineages. Using the Unifrac significance test and the P-test showed significant differences between strain populations and geographic regions. The degree of those differences, however, varied among the genes analyzed and the countries included.
Conclusion: The data indicate that the salivary microbiome may hold geographic signatures for providing new perspectives on unsolved human migration and evolution patterns—an issue of medical, social and anthropological importance.

PROBIOTICS IN THE CONTROL OF THE INCIDENCE AND SEVERITY OF *CLOSTRIDIUM DIFFICILE* INFECTIONS AT A COMMUNITY HOSPITAL

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Objective: A quasi-experimental cohort study evaluating the impact of adding a probiotic (BIO-K$^{+®}$: *Lactobacillus acidophilus* CL1285® and *L. casei* LBC80R®) to existing *Clostridium difficile* infection (CDI) standard preventative measures (SPM) during 7 years.
Background: In 2003, hospitals in Quebec experienced an increase in the mortality rate due to an outbreak of CDI. Pierre-Le Gardeur Hospital (PLGH), despite an existing CDI prevention program, experienced an increased incidence from 10 to >25 cases per 1000 patient admissions (PA). Severe cases and deaths related to CDI increased from zero to an average of 5.1 cases per 1000 PA.
Methods: Observations from 31832 hospitalized patients divided into 4 phases. Outcomes: CDI incidence and recurrence rate, severe CDI cases incidence and safety of the probiotic. Phase 1 (2003/08-2004/01) included 1580 patients and measured the baseline impact of reinforcing a bundle of CDI SPM. Phase 2 (2004/02-2004/03) included continuation of SPM with the addition of administration of a probiotic to all patients under antibiotics. Phase 3 (2004/05-2005/08) included the same interventions after a move to a new hospital facility. During phase 2 and 3, 4968 patients received a 50 to 60 x 10^9 cfu daily dose of oral probiotic. In the course of phase 4 (2005/09–2011/03), an additional 25284 patients were submitted to the same regimen and outcome data were submitted to a provincial surveillance program.
Results: CDI incidence at PLGH decreased from ≥18 cases in February 2004 to <5 cases per 1000 PA at the end of August 2005. 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 at PLGH compared to 8.50 cases per 10000 patient-days in equivalent hospitals in Quebec.
Conclusions: The addition of a prophylactic probiotic to a bundle of SPM resulted in a reduction in CDI rates. No serious events were observed.

SXVII-3

POLYSACCHARIDE BIOSYNTHESIS LOCI DIVERSITY AMONGST *BACTEROIDES FRAGILIS* ISOLATES

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The production of diverse and variable surface polysaccharides is associated with *Bacteroides fragilis* colonisation of humans in health and disease. *In silico* analyses of complete genome sequence data using the Artemis Comparison Tool (Wellcome Sanger Institute), combined with monoclonal antibody labelling and immunofluorescence microscopy, reveals considerable amongst strain diversity in polysaccharide (PS) biosynthesis loci in *B. fragilis*. Of ten divergent PS-associated loci apparent in each individual strain, none is similar between the two fully sequenced strains NCTC 9343 (origin UK) and 638R (origin USA). A fully sequenced strain from Japan, YCH46, shares one locus with NCTC 9343 which is confirmed by labelling with specific antibody, and a second different locus with 638R. This makes a total of 28 divergent PS biosynthesis loci amongst the three strains. Study of further isolates confirms this unprecedented level of amongst strain diversity of PS loci. The zwitterionic amino sugar-containing NCTC 9343 PSA, which is involved in abscess formation and which also protects animals from experimental colitis, is not present in either strain 638R or YCH46. The presence of putative aminotransferase and dehydrogenase genes within other loci, however, may be indicative of the production of polysaccharides with similar properties. On this basis, four of the 638R PS loci may produce a different set of abscess-inducing microcapsules.

The multiple and diverse PS biosynthesis loci in *B. fragilis* indicates a strong selective pressure in favour of PS variation within the human gastrointestinal (GI) tract. This is likely to have arisen from divergence of operons over evolutionary time combined with more rapid changes arising from horizontal transmission amongst cells representing a pan-genome with an extensive pool of different PS loci. It may be that a major driver of amongst-strain PS locus diversity, within-strain surface protein and PS diversity generated by DNA inversion and extensive DNA restriction and modification, is the prevention of bacteriophage adsorption and infection within the GI tract.

A UNIQUE HOMOLOGUE OF EUKARYOTIC UBIQUITIN PRODUCED BY *BACTEROIDES FRAGILIS* WITH THE POTENTIAL FOR SIGNIFICANT HOST INTERACTION

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The Bacteroidetes are predominant members of the normal human gastrointestinal tract microbiota. *Bacteroides fragilis* represents ~10-15% of the *Bacteroides* spp. present in faeces, with estimates of between 10^{11}-10^{12} cells/g by culture. In the complete genome sequences of *B. fragilis* NCTC9343 and 638R, we discovered a gene, *ubb,* the product of which (BfUbb) has 63% identity to human ubiquitin and cross-reacts with antibodies raised against bovine ubiquitin. The sequence of *ubb* is closest in identity (76%) to the ubiquitin gene from a Migratory Grasshopper entomopoxvirus, suggesting acquisition by inter-kingdom horizontal gene transfer. A protein with such significant similarity to eukaryotic ubiquitin has not been discovered previously in prokaryotes. Here, we show that some, but not all, *B. fragilis* clinical isolates, contain *ubb*. BfUbb has a predicted signal sequence; a processed form is detectable in whole cell extracts and, also outer membrane vesicles (OMV). OMV may act as a delivery system providing a mechanism by which *B. fragilis* interacts with the host. We also show that purified recombinant BfUbb inhibits *in vitro* ubiquitination and is able to covalently bind the human E1 activating enzyme, suggesting it could act as a suicide substrate *in vivo*. These data indicate that the gastrointestinal tract of some individuals could contain a significant amount of aberrant ubiquitin with the potential to interfere with eukaryotic ubiquitin activity.

SXVII-5

EFFECT OF ANTIRETROVIRAL TREATMENT ON SELECTED GUT MICROBIOTA IN SOUTH AFRICAN HIV POSITIVE PATIENTS

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HIV-positive patients have a high risk of developing diarrhoea, either due to the HIV-infection or the use of antibiotics disrupting the GIT microbiota. This study aimed to establish whether there were changes in the diversity/abundance of dominant/sub-dominant gastrointestinal bacterial groups, present in the faeces of HIV-positive patients, before initiating and during 6 months antiretroviral (ARV) treatment, and to determine whether targeted probiotic supplementation could provide additional supportive care. The intestinal microbial composition, with respect to total *Eubacteria*, *Bifidobacterium, Lactobacillus*, *Bacteroides/Prevotella*, *Clostridium coccoides, Clostridium leptum,* and *Escherichia coli* was characterised using PCR-DGGE (diversity) and qPCR (abundance), in 12 HIV-positive and 12 HIV- negative donors. When compared to the HIV-negative control group, the HIV-positive patients showed (i) less of the Eubacterial and *C. leptum* groups, (ii) marked changes in the diversity of *Bifidobacterium* and *Lactobacillus* communities, and (iii) a stronger predominance of *B. adolescentis* and a reduction of *B. longum* during ARV treatment. Additional antibiotic administration caused a further significant reduction in the diversity of the *Bifidobacterium* and *Lactobacillus* groups. This is the first study to report the levels of bacterial species in the GITs of South African HIV-positive patients, and to show that ARV treatment over a 6 month period does not restore the normal HIV negative microbiota profiles in spite of major improvements in the CD4 count. Probiotic administration could be considered to assist in normalizing the gut microbiota.

GUT MICROBIOTA IN PRETERM INFANTS THROUGHOUT THE FIRST YEAR OF LIFE

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Gut microbiota establishment is an important step in the maturation of intestine. Delayed establishment has been reported in preterm neonates, that is a risk factor for gastrointestinal diseases. We aimed at describing this gut microbiota establishment throughout the first year of life. This monocentric prospective longitudinal study enrolled 83 preterm infants. Fecal samples were collected at 1 week (wk), 1, 3, 6, and 9-12 months of life and analysed using culture and culture-independent methods. Colonization pattern was depending on gestational age (GA). At 1 month a delayed colonization by staphyloccocci, enterococci, and enterobacteria was observed mainly in infants born at a GA <28 wks. As far as anaerobes were concerned, few infants were colonized by bifidobacteria and *Bacteroides* whatever their GA, although clostridia were more often found. At 3 months, almost all infants were colonized by the aerobic genera. At 6 months almost all infants born at a GA >33 wks were colonized by bifidobacteria and *Bacteroides* by contrast with those born at <33 wks. At 9-12 months, a delayed in colonization by *Bacteroides* was still observed in infants born at a GA <33 wks. Differences in species colonizing the infants were also observed. Less than two third of the infants born at GA <33 wks were colonized by *Escherichia coli* at a median age of 111 days for infants born at a GA <28 wks, and 31 days for those born at a GA 28-<33 wks. However, in all infants, colonization by *Enterobacter* sp, *Klebsiella* sp, *Citrobacter* sp was frequent. Our data confirm the abnormal colonization in preterm infants. The bacterial pattern depends on the GA. Moreover, we show that delayed colonization by bifidobacteria and *Bacteroides*, known for their health promoting properties, can be observed up to 1 year of life in infants born at a low or very low GA. This may contribute to the development of later diseases such as but may be also for later diseases such as allergy, inflammatory bowel diseases, or obesity.

This study is supported by the French National Research Agency.

Anaerobe 2012

1445 POSTER SESSION I: COLONIC MICROBIOTA

Posters will be presented in Poster Session I
Friday, June 29 1445-1545.

IS THERE ANY DIFFERENCE IN INTESTINAL *BIFIDOBACTERIUM* SPECIES IN HEALTHY TURKISH CHILDREN—COMPARING WITH TURKISH CHILDREN DIAGNOSED WITH ASTHMA AND ALLERGIC DERMATITIS

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Bifidobacteria are a major component of the human and animal gastrointestinal tract. The purpose of this study was to determine at species level the intestinal bifidobacterial colonisation in children diagnosed with asthma and allergic dermatitis and compare to the results of healthy children.

Stool specimens taken from 17 children under three years, 12 of them diagnosed with asthma and 5 diagnosed with allergic dermatitis and stool samples of 17 healthy children under 3 years were collected immediately on trypticase phytone yeast extract (TPY) broth and incubated 72 hours on anaerobic conditions obtained with Anaero-Gen (Oxoid & Mitsubishi Gas Company) in anaerobic jars (oxoid). One or two loops of each TPY broth were subcultured on Wilkins Chalgren Agar (WCA), on Man Rogosa Sharp (MRS) agar and on Trypticase Phytone Yeast extract (TPY) agar and incubated an additional 72 hours on anaerobic conditions. Identification of *Bifidobacterium* colonies was made according to the Gram stain, the morphologic characteristics and PCR. Specific primers prepared for *B.longum, B.breve,B.adolescentis, B.bifidum*, *B.infantis*, *B.lactis*, *B.catenulatum* and *B.pseudocatenulatum* (Ella Biotech GmbH, Germany) were used .

B.bifidum and *B.longum* were isolated from 6 (50%) and 2 (16.6%) asthmatic children respectively and were isolated from 3 (60%) and 4 (80%) children with allergic dermatitis. These species were found in all healthy children. *B.adolescentis* (0%), *B.infantis* (0%), *B.lactis* (0%), and *B.catenulatum* (0%) were not isolated from both groups *B.pseudocatenulatum* and *B.breve* were the most frequently isolated species in both groups *B.breve* was isolated in 10 (83.%) asthmatic and 5 (100%) allergic children and in 14 (82%) healthy children. *B pseudocatenulatum* was isolated in 8 (66.%) asthmatic and 4 (80%) allergic children and in 12 (70.5 %) healthy children .

According to our results, we concluded that data of advanced researches with high number of specimens could give more relevant results. Eespecially the decreased number of *B.bifidum* and *B.longum* in asthmatic children's intestinal flora could play an important role that needed to be enlightened.

Supported by Istanbul University Research Found., Project No. 13038

PI-2

PROTEOMIC ANALYSIS OF *BACTEROIDES FRAGILIS* OUTER MEMBRANE UNDER STRESS INDUCED BY BILE SALTS

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Bacteroides fragilis is the most common anaerobic bacteria isolated from infectious processes. Several virulence factors contribute to the pathogenic nature of this bacterium, among them the ability to tolerate oxygen and the high concentration of bile salts. Bile salts have a similar activity to detergents and may lead to membrane permeabilization and cell death. Tolerance to bile salts is essential for the survival of this bacterium and may as well be related to pathogenicity. This ability may be related to the expression of outer membrane proteins, which have been shown to act as efflux pumps for toxic substances, such as antibiotics and aromatic compounds. The bacterial outer membrane is the first line of contact of the bacteria with the environment, and its composition is highly variable and may be quickly restructured to adapt to environmental pressures. The current works seeks to identify outer membrane proteins in *B. fragilis* associated to the stress induced by bile salts. The outer membrane of *B. fragilis* strain 638R was isolated after growth either in the presence of 2% bile salts or without bile salts. The membrane fractions were separated on SDS-PAGE and analyzed by ESI-Q-TOF tandem mass spectrometry. Our preliminary results showed the expression of certain proteins associated to the presence or absence of bile salts that are related to cellular functions such as transport, nutrient uptake and protein-protein interactions. An insertional mutation was performed in the gene coding for the BF0752 protein, which is expressed only in the presence of bile salts for phenotypic analysis under bile salt stress. Further experiments are being performed to get a better understanding about the role of outer membrane proteins in the pathogenicity and antimicrobial resistance of *B. fragilis*.

Financial support: CAPES, MCT/CNPq, MCT/PRONEX/Faperj and Faperj.

THE INFLUENCE OF *FUSOBACTERIUM NUCLEATUM* IN SYNERGISTIC INFECTIONS

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The intestinal microbiota is a dynamic ecosystem of emerging importance to human health. Bacterial species within this ecosystem have co-evolved with their host to allow for the maintenance of homeostasis and to avoid imbalances in microbial populations which in turn may lead to a diseased state in the host. The search for members of the microbiota that are capable of driving inflammatory responses in the host is a current research focus. In this respect *Fusobacterium nucleatum* (*Fn*) is a potential candidate member of the normal intestinal microbiota with pro-inflammatory attributes, and this species has recently been specifically associated with inflammatory bowel disease and colorectal cancer. *Fn* is an invasive pathogen that is also known to co-aggregate with a wide variety of different bacterial species. It has been shown that, through such co-aggregation, *Fn* can directly facilitate internalization of other normally non-invasive bacterial species *in vitro*. Such a phenomenon offers a potential mechanism whereby luminal bacterial species can come into direct contact with the host epithelial immune system, which may in turn contribute to an inflammatory response. If this is the case, then the involvement of *Fn* in disease could be a function of the microbes to which it aggregates, rather than a direct result of its own virulence determinants. We have found that different *Fn* isolates have distinct binding trophisms for particular secondary bacterial binding partners isolated from the human gut. This study aims to examine the implications of such co-aggregation on co-invasion by *Fn*, and the resulting downstream effects on the stimulation of inflammatory markers and/or enhancement of invasion capabilities of bound bacterial species.

PI-4

ANTIMICROBIAL PROFILES OF *BACTEROIDES* AND *PARA-BACTEROIDES DISTASONIS* ISOLATED FROM A BRAZILIAN INTENSIVE CARE UNIT

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The genus *Bacteroides* is one of the major microbial groups that form the intestinal microbiota. Although a minor component in this niche, *Bacteroides fragilis* is the principal anaerobic species associated with clinical infections, such as intra-abdominal and bloodstream infections. Two of the major species in this niche, *B. thetaiotaomicron* and *Parabacteroides distasonis,* have been considered as potential pathogens and reservoirs for antibiotic resistance genes. The aim of the present study was to assess the antimicrobial resistance rates of different anaerobic species in a cohort of patients admitted to an intensive care unit of a university hospital in Rio de Janeiro. Rectal swab specimens were collected weekly from admission to discharge, from April 2007 to April 2008. Isolates were identified by conventional biochemical testes, and antimicrobial susceptibility was determined by agar dilution (CLSI) and E-test. The presence of 5 resistance genes was investigated by PCR. In total, 537 specimens were collected from 193 patients, and 123 anaerobic isolates were obtained from 65 (34%) patients. Most frequent bacterial species were *B. fragilis* (26%), *B. thetaiotaomicron* (25%), *P. distasonis* (20%), *B. vulgatus* (14%) and other *Bacteroides* species (15%). All isolates were susceptible to imipenem, 4 (3%) showed decreased susceptibility to amoxicillin/clavulanate (MIC 8 mg/L) and 3 (2%), to metronidazole (MIC 4 mg/L). High resistance prevalences were observed for tetracycline (76%), clindamycin (50%), moxifloxacin (26%), cefoxitin (21%) and cefotaxime (20%). Multidrug-resistance (co-resistance to at least three different antimicrobials) was observed in 33% of the isolates, but varied within different species: 71% among *B. thetaiotaomicron*, and 9% among *B. fragilis*. The prevalence of resistance genes were: *tet*Q, 85%; *cep*A, 33%; *erm*F 12%; *cfi*A, 4%; and *nim* 2%. The present study revealed high rates of resistance to several antimicrobial agents among isolates of the commensal microbiota. Important resistant determinants were highly prevalent.

Financial Support: CNPq, PRONEX-FAPERJ

THE PLASMINOGEN-BINDING PROTEIN (BFP60) IS IMPORTANT IN *BACTEROIDES FRAGILIS* PATHOGENICITY

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B. fragilis is an anerobic bacterium and a member of the gastrointestinal flora of humans, but is also cause a severe intra-abdominal infection. Recently, a putative plasminogen-binding protein, Bfp60 located in the outer membrane, was identified in *B. fragilis*. After a few analyzes, our studies showed that Bfp60 could not only recognize, but also convert Plasminogen (Plg) into Plasmin. Indiscriminate activation of plasmin can cause tissue damage and can also transform a nonproteolytic bacterium into a proteolytic one. Thus, the aim of this work was to construct insertion mutants using the clinical strain 638R, to demonstrate that Bfp60 is important to the virulence of the species in *in vitro* and *in vivo* assays. Briefly, oligosaccharide primers were designed to amplify an internal region of the gene *bfp*60 (512 bp) and the *Sph*I/*Pst*I bfp60 fragment of pGEM T easy was cloned into *Sph*I/*Pst*I -digested pFD516 and the new construct mobilized from *Escherichia coli* DH10B in *B. fragilis* strain (BE1) by aerobic triparental filter mating. All mutants were selected on BHIS agar plates containing 100μg/mL Gentamicin and 10 μg/mL Erythromycin. Mutants were analyzed for Plg and laminin-1 adhesion; and conversion of Plg into plasmin. Our results showed that mutants for the *bfp60* gene could not strongly adhere to Plg and laminin-1 anymore and they also lost the ability to convert Plg into plasmin. Since, during the peritonitis the coagulation and fibrinolytic cascade are up regulated, with the formation of fibrin in the abdominal cavity leading to the intra-abdominal abscess, *in vivo* assays using the abscess formation model in mice are been performed to check if mutants lost their ability to form abscess. Certainly, mutants for the putative plasminogen-binding protein will help to understand if the Plg recognition and activation can contribute with the pathogenicity of *B. fragilis*.

Financial support: Pronex-FAPERJ, CNPq, CAPES.

PI-6

MUCIN DEGRADATION BY CLINICAL ISOLATES OF *BACTEROIDES FRAGILIS* FROM GROOTE SCHUUR HOSPITAL, CAPE TOWN

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Bacteroides fragilis is an anaerobic bacterium that is present in the human gastrointestinal tract (GIT). Through microbial-host interactions, the bacteria may function as either a commensal or pathogen. The mucosal layer of the GIT is crucially important for colonisation, nutrition and virulence of this bacterium. Pathogenicity is initiated when the organism escapes though discontinuities of the mucosal layer which leads, ultimately, to bacteraemia and abscess formation. In this study, 23 clinical isolates of *B. fragilis* from Groote Schuur Hospital (GSH) were tested for the ability to degrade mucin – the major component of the GIT mucosal layer. A mucin plate degradation assay showed that all of the 23 strains were able to degrade porcine gastric mucin. Growth curve analysis revealed that mucin can be utilized by *B. fragilis* as the sole carbon source enabling growth levels comparable to those of glucose containing defined media and complete medium. Mucin zymography of the cell free extract, outer membrane protein fraction and the extracellular fraction identified a ~90 kDa and a ~115 kDa protease, both of which are capable of degrading mucin *in vitro*. Further characterisation of these proteins will provide insight into the molecular interactions between *B. fragilis* and the mucosal layer.

RESISTANCE TO HEAVY METALS OF INTESTINAL SPECIES FROM HUMAN AND ANIMAL ORIGIN

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Bacteroidales species are often associated to opportunistic mixed infections, and these microorganisms are able to develop resistance to several antimicrobials. The human and animal contamination with mercuric and cadmium ions is commonly observed, particularly, because of inadequate planning to eliminate the metallic residues from environment. In this study, the susceptibility and the presence of resistance genes to metals of *Bacteroidales* species isolated from children and calves with diarrhea were evaluated. The susceptibility to heavy metals was performed by using an agar dilution method with a Wilkins-Chalgren agar. The metals were: zinc sulfate ($ZnSO_4$), cadmium sulfate ($CdSO_4$), mercuric chloride ($HgCl_2$) and cobalt sulfate ($CoSO_4$). Plasmid DNA from resistant strains was obtained by using commercial kits. Plasmid-positive strains were treated with ethidium bromide. When plasmid loss was observed, the susceptibility was again evaluated to verify the resistance values. Species-specific primers were used to detect the presence of genes *mer*A, *mer*R, *czc*A and *czc*D. A high bacterial resistance to metals was observed (from 77% to 100%). Only one *B. vulgatus* from human origin displaying 3.0 and 5.0 kb plasmids, and one *B. vulgatus* from animal origin showing a 4.0 kb plasmid were observed. The animal strain *B. vulgatus* (B2-3i) displayed a decreased MIC value to $CdSO_4$, from 32 to 8 mg/mL, and the *czc* gene was observed. No *Bacteroidales* species harbored the *merA, merR, czcA* or *czcD* genes on chromosome. The presence of *czc* gene in intestinal *Bacteroidales* seems to be the first report in literature, specifically in *B. vulgatus.* Further studies to characterize the Czc efflux system in *Bacteroidales* will be necessary.

PI-8

PROTEOMIC ANALYSIS OF THE OUTER MEMBRANE VESICLES PRODUCED BY *BACTEROIDES FRAGILIS*

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Bacteroides fragilis, an important Gram-negative commensal of the human gastro-intestinal tract, produces a large quantity of outer membrane vesicles (OMV), which may deliver effectors to host epithelial cells and/or other bacteria associated with the mucosal layer. Effectors could include components that facilitate quelling of the host immune response, or toxic compounds that damage competing bacterial species. These vesicles are also of medical interest, potentially playing a role in development of inflammatory bowel diseases, such as Crohn's disease. Proteomic studies of OMV from other Gram-negative bacteria reveal components from all subcellular compartments, but primarily the outer membrane and periplasm, and have suggested a sorting mechanism for incorporation into vesicles. In the current study, the "snapshot" proteome of OMV from *B. fragilis* is elucidated and compared with that of a whole cell extract.

OMV and cells were separated from cultures of *B. fragilis* NCTC9343; the concentration of both vesicles and cells was determined by Bradford assay, and the purity of vesicles analysed by dynamic light scattering. Samples of cells and vesicles were analysed by high-purity liquid chromatography and MS-MS with an LTQ-Orbitrap mass spectrometer (Thermo Scientific). Peptides were mapped to the predicted products of the *B. fragilis* genome using the Progenesis LC-MS software (Nonlinear Dynamics). The majority of proteins identified in the current study originated from the outer membrane or periplasm; the function and diversity of these proteins will be discussed with respect to the potential role of *B. fragilis* in host epithelial cell manipulation and the potential role of OMV in human health and disease.

INCREASED ANTIMICROBIAL SENSITIVITY INDUCED BY OVEREXPRESSION OF MULTIPLE ANTIBIOTIC RESISTANCE REGULATOR PROTEINS IN *BACTEROIDES FRAGILIS*

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Among the most important species of anaerobes associated with humans, *Bacteroides fragilis* plays a role in the gastrointestinal tract, both as a member of the microbiota and as an opportunistic pathogen, causing mainly abscesses and bacteremia. *B. fragilis* produces several virulence factors, such as fimbriae and agglutinins, capsular polysaccharide complex (CPC), LPS and a variety of enzymes. *B. fragilis* also has significant oxygen tolerance and resistance to various antimicrobials, which can further contribute to the maintenance and progression of infection. MarR family proteins are transcriptional regulators that bind directly to DNA and control various physiological processes in bacteria and archaea, including the oxidative stress response, antimicrobial resistance and production of virulence factors. We found three MarR homologues in *B. fragilis* strain 638R. Thus, our study aimed at the construction of two mutants expressing the proteins, referred here as BmrR I and II (BF3296, BF3863), under the control of an inducible promoter for phenotypic analysis. For the expression of proteins, the *bmrR I* and *II* genes were amplified by PCR, where primers were fitted with sites of restriction enzymes and cloned in a maltose-inducible vector, pFD1045. The plasmid containing the gene was inserted by electroporation into *E. coli* and then transferred to *B. fragilis* by triparental matting. The two strains of *B. fragilis* over-expressing proteins were tested for antimicrobial resistance and used in trials of exposure to oxygen. The engineered strains displayed more sensitivity to several antimicrobials tested, such as tetracycline, ciprofloxacin, imipenen and tigecycline when grown in defined medium with maltose as sole carbon source. The over-expression of MarR-like proteins also reduced the ability of *B. fragilis* to survive oxidative stress. Our results indicate that over-expression of MarR-like proteins inhibits the expression of genes, most likely efflux pumps, involved in detoxification of the bacterial cell.

PI-10

FLUORESCENT PROTEINS USED TO MONITOR BACTERIOFERITIN GENE EXPRESSION IN *BACTEROIDES FRAGILIS*

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Reporter genes are used as fusion proteins with regulatory regions of genes for visualization of its expression or activity. The traditional reporter genes based on Green Fluorescent Protein (GFP) are oxygen dependent, which prevents its use in anaerobiosis. The Flavin Mononucleotides based Fluorescent Proteins (FbFP) were developed to overcome such restrictions and can be used in anaerobic systems, such as studies with *Bacteroides* spp. The *B. fragilis* species has been distinguished by its clinical importance and the expression of serious aggression factors. Among these pathogenesis determinants, iron acquisition mechanisms stands out, primarily those which are involved with the degradation of ROS. Iron is stored in protein structures called Bacterioferritins (Bfr), which can protect the bacteria from excess of this ion. The purpose of this work was to standardize a study protocol using BS2 protein as reporter gene for Bfr expression in *B. fragilis*. In this work, pGem – T easy® plasmid, harboring the BS2 protein gene was digest with the restrictions enzymes *Sac*I and *Bam*HI, cloned into constitutive expression plasmid pFD340 and inducible expression plasmid pFD1045, in *E. coli* and transferred to the *B. fragilis* 638R strain by triparental mating, where they were selected by the erythromycin resistance expression. The selected strains were analyzed by confocal microscopy, for the expression of the expected fluorescence. Then, we analyzed the expression and regulation of the *B. fragilis bfr* gene (BF1214). The region 208bp upstream of *bfr* gene, which contains a *B. fragilis* promoter as previously described in the literature, was cloned *in tandem* with the *BS2* gene into the pFD288 plasmid. This plasmid doesn't have a regulatory region and the BS2 expression is controlled by the *bfr* promoter. This way, we can observe the influence of environmental factors, namely iron availability, on the gene expression.

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ANTIBIOTIC RESISTANCE OF CLINICAL ISOLATES OF *BACTEROIDES FRAGILIS*

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Bacteroides fragilis, an anaerobic gut commensal and opportunistic pathogen, is a leading cause of anaerobic abscesses and bacteraemias. Treatment of infections is complicated by the emergence of resistance to several of the antibiotics, specifically metronidazole, used in the clinical setting. This study aimed at examining the levels of antibiotic resistance of 23 *B. fragilis* strains isolated at Groote Schuur Hospital, Cape Town, and determining the metronidazole resistance mechanism to evaluate the clinical risk of the spread of drug resistance. The minimum inhibitory concentration of the strains was established using the E-test method. Eight percent of the strains were highly resistant to metronidazole, imipenem and cefoxitin. Tetracycline was the least effective with 65% of strains showing resistance. All strains were sensitive to clindamycin, and strain *B. fragilis* GSH 15, showed multidrug resistance to metronidazole, imipenem, cefoxitin and tetracycline. Antibiotic resistance gene screening, by PCR, did not detect any *nim* or *cfxA* genes. However, it did reveal the presence of the *tetQ* and *cfiA* genes in 69% and 13% of the strains, respectively. One metronidazole sensitive strain showed spontaneous development of resistant colonies. Published metronidazole resistance mechanisms involve drug inactivation due to the nitroreductase activity encoded by the *nim* genes. As no *nim* genes were detected an alternative mechanism may be due to disruptions in the bacterial pathway converting pyruvate to lactate via lactate dehydrogenase (LDH) and the associated change in electron flux can prevent intracellular metronidazole activation. All the metronidazole resistant strains showed increased LDH activity indicating the involvement of this pathway in metronidazole resistance. Multiple sequence alignment of the pyruvate-ferrodoxin oxidoreductase gene (PFOR) indicated that only the multidrug resistant strain, GSH15, had an altered sequence. The risk of metronidazole resistance spread via *nim* is, therefore, not significant in this study. Continued surveillance of metronidazole resistance is thus recommended.

PI-12

EXAMINATION OF β-LACTAM RESISTANCE MECHANISMS OF *BACTEROIDES* STRAINS

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Objectives: We aimed to study the susceptibilities of the main applicable beta-lactam drugs of clinical *Bacteroides* strains and to correlate these results with the beta-lactamase activities, with the *cepA* gene and its genetic environment together with the *cfxA* and *cfiA* gene contents.
Methods: Agar dilution was applied to determine the susceptibilities of ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoxitin and imipenem of 43 *Bacteroides* strains. The *cepA*, *cfxA* and *cfiA* genes were detected by PCR. PCR methods were applied after a bioinformatic analysis of known *B. fragilis* genomes that harbour the *cepA* gene to examine its genetic environments. We also looked for the presence of activating insertion sequence (IS) elements (IS*1224* and IS*614*) in the upstream regions of *cepA*. Beta-lactamase activities were recorded using nitrocefin.
Results: Of the 43 *Bacteroides* strains 28 *B. fragilis* (n=34, 82.4%) and 4 non-fragilis *Bacteroides* (n=9, 44.4%) harboured the *cepA* gene. 7 (16.3%) were positive for *cfxA* and 2 *B. fragilis* (7.1%) for the *cfiA* genes. All but one of the 28 *cepA* genes were located on a 1745 bp chromosomal fragment by PCR detection, however, 7 of these could be localized on chromosomal loci other than those for *B. fragilis* NCTC9343 and 638R. None of the *cepA* genes could be demonstrated to be activated by the IS*1224* or IS*614* elements. 15 strains produced beta-lactamase activities higher than 10mU/mg protein of which 9 were *cfxA* or *cfiA*-positive. The correlation coefficient between the beta-lactamase activities and the ampicillin MICs was 0.62 ($p<0.000$).
Conclusions: The *cepA* genes of *Bacteroides* strains are usually found on a 1745 bp chromosomal element without IS activation and low beta-lactamase production. For higher beta-lactamase activities the presence of other genes (*cfxA* and *cfiA*) are usually required.

HUMAN GASTROINTESTINAL MICROBIOME OF INDIGENOUS PERUVIAN COMMUNITIES

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The National Institute of Health launched the Human Microbiome Project in 2007 with the goal of characterization of the human microbiome and its influences on human health and disease. To date nearly all studies of this type have been preformed in 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 order to better create a generalized core human gastrointestinal microbiome, data from developing nations should also be considered as over 80 percent of the global population resides in developing nations. Our study is unique in that we study the human gastrointestinal microbiome of individuals from geographically remote, traditional native communities in the developing nation of Peru.
We present data inferred from fecal samples collected from two distinct Peruvian communities. Samples were collected from both a traditional Matsés community from the Loreto Region and a traditional African American community from along the coast of Ica, Peru. While we expect these communities to harbor some degree of the gastrointestinal tract microbial co-dominance by the phyla Firmicutes and Bacteroidetes, commonly observed in industrialized nations, we believe that gastrointestinal microbes from these remote geographic regions will be unique in phylotypes and functional roles and harbor novel microbes. Distinct in culture, diet and lifestyle these two communities will aid in our understanding of the traditional indigenous gastrointestinal microbiome. Cultivation and characterization of representative taxa from these communities is essential in gaining a more complete understanding of the ecology of the gastrointestinal tract in addition to revealing microbes atypical of those found in industrialized nations.

PI-14

US NATIONAL SURVEY ON THE SUSCEPTIBILITY OF *BACTEROIDES FRAGILIS:* RESISTANCE BY SPECIES AND CENTERS FOR THE YEARS 2006-2010

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Objective: Analysis of five years data on *B. fragilis* group isolates was used to determine the association of antibiotic resistance with species and medical center by examining a significant number of isolates referred by US centers.
Methods: The MICs of 2295 isolates referred by 8 medical centers were determined by agar dilution (CLSI M11-A7). The antibiotics were: imipenem, meropenem, ertapenem, ampicillin:sulbactam (A/S), piperacillin:tazobactam (P/T), cefoxitin (Fox), tigecycline (Tig), moxifloxacin (Mox), clindamycin (Clnd), linezolid (Lin), chloramphenicol and metronidazole.
Results: Table 1 shows the highest resistance rates and their association with species and center compared to the overall resistance rates combined.

Agent	Species	Center	Highest R	Overall R
A/S	*distasonis*	Duke	27.3%	15.7%
Fox	*ovatus/dist*	Duke/Mayo	27.3/22.9%	13.9/15.3%
Mox	*vulgatus*	NY/Michigan	84.6/70%	63.1%
Tig	*ovatus*	Duke/Loyola	13.2/12%	7.4%
Clnd	*vulgatus*	NY/Duke	61.5/54.3%	42.7%

The carbapenems and P/T were the most active agents; resistance, when observed, was mostly associated with *B. fragilis.* Two isolates from U Michigan were metronidazole-resistant. Five isolates from 4 different centers showed elevated MIC to chloramphenicol, 16 mg/L. For most centers resistance to Mox and Clnd exceeded 50% and 30%, respectively.
Conclusions: Resistance among the *B. fragilis* group continues to increase, presumably due to the selective pressure of specific antibiotics.

Variation of resistance between centers points out the need for caution in over generalizing national results and underscores the need for information regarding susceptibility on a center by center basis.

Resistance trends demonstrate the need for speciation since there are profound differences in susceptibility among species and agents.

INFANT GUT MICROBIOTA WITH DOMINANCE OF *BIFIDOBACTERIUM* SPP. AND *BACTEROIDES* SPP. IS PROTECTIVE AGAINST COW'S MILK ALLERGY DESPITE IMMATURE ILEAL T CELL RESPONSE

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Faecal commensal microbiota in healthy infant displays a large abundance in *Bifidobacterium* spp. and *Bacteroides* spp. Although some studies indicated an association between these two genera and allergy, it still remains a subject of debate. Using a gnotobiotic mouse model of cow's milk allergy, we aimed at investigating the impact of an infant gut microbiota mainly composed of *Bifidobacterium* spp. and *Bacteroides* spp. on immune activation and subsequent effect on allergic manifestations. The dominance of *Bifidobacterium* and *Bacteroides* was preserved in gnotobiotic mice when looking at faecal samples by plating and pyrosequencing. The transplanted microbiota failed to restore an ileal T-cell response similar to the one observed in conventional mice. This may be linked to the low bacterial translocation into Peyer's patches in gnotobiotic mice. Allergic response was then monitored in germ-free, gnotobiotic and conventional mice after repeated oral sensitizations with whey proteins and cholera toxin. Gnotobiotic and conventional mice displayed lower drop of rectal temperatures upon oral challenge with β-lactoglobulin, lower plasma mMCP-1 and anti-β–lactoglobulin IgG1 than germ-free mice. Interestingly, the *foxp3* gene was highly expressed in the ileum of both gnotobiotic and conventional mice that happened to be protected against cow's milk allergy. This work shows for the first time that a transplanted infant microbiota mainly composed of *Bifidobacterium* and *Bacteroides* has a protective impact on sensitization and food allergy despite altered T cell response in the ileum.

PI-16

A FLOTILLIN-LIKE PROTEIN MODULATES METRONIDAZOLE RESISTANCE IN *BACTEROIDES FRAGILIS*

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There is an increase in the occurrence of *Bacteroides fragilis* clinical isolates showing resistance to metronidazole. A number of these isolates do not harbour any known *nim* gene, which is the most common metronidazole resistance determinant in *B. fragilis*. In order to identify possible alternative metronidazole resistance mechanisms, a *B. fragilis* 638R random transposon mutant bank was generated and a metronidazole resistant mutant isolated and characterised. The *B. fragilis* mutant contained a transposon insertion between the genes of an operon encoding a hypothetical protein (BF638R_0902) and a flotillin-like gene (BF638R_0901). Transcriptional studies, as demonstrated by quantitative reverse transcription PCR, showed that the transposon insertion interrupted the operon, and that the flotillin gene transcript was present in reduced quantities relative to the wild-type. Bioinformatic analysis showed that the BF638R_0902- BF638R_0901 operon was conserved in all members of the *Bacteroides fragilis* group. Increased resistance to metronidazole was also demonstrated in an insertional mutant of BF638R_0901 (flotillin-like) gene. This study has revealed a previously unknown component of the metronidazole resistance phenotype in *B. fragilis*. The possible role of this membrane-associated protein in relation to metronidazole resistance through the inhibition of drug uptake is explored.

1445 *POSTER SESSION I:* ORAL MICROBIOTA & DISEASE

Posters will be presented in Poster Session I
Friday, June 29 1445-1545.

PI-17

ANTIMICROBIAL ACTIVITY OF VANILLIN AGAINST ORAL BACTERIA

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Several methods of interfering with the accumulation of bacteria in dental biofilm have been tried in order to prevent oral diseases. Natural compounds have already shown interesting antibacterial and anti-adhesive activities. The purpose of this investigation was to assess the antimicrobial activity of vanillin at different concentrations against oral bacteria in their planktonic and biofilm-embedded states. Four bacterial species involved in dental decays (*Streptococcus mutans*, *Streptococcus sobrinus, Actinomyces viscosus*, *Lactobacillus rhamnosus*), and two species that represent pathogenic flora of periodontal diseases (*Porphyromonas gingivalis* and *Fusobacterium nucleatum*) have been tested.

The antimicrobial activity of synthetic vanillin was examined by determining the MIC and MBC using the macro dilution broth technique. The anti adhesive activity of vanillin was tested with two methods: the inhibition of adherence of growing cells to a glass surface, and the biofilm formation on hydroxyapatite: A biofilm containing all the tested species was developed on hydroxyapatite discs coated with human pasteurized saliva. Each experiment was repeated three times.

Our results demonstrate dose-dependent and species-dependent anti bacterial effect of vanillin against all the tested bacteria. The inhibitory effect on bacterial adherence to glass surface of the different concentrations of vanillin is species-dependent. The formation of biofilm on hydroxyapatite is not totally inhibited, but the bacterial mass is significantly decreased. Vanillin shows interesting inhibitory activities on growth and adhesion of species involved in oral diseases. Related to other natural compounds active against the same bacteria, like polyphenols, its production is less expensive, it has a nicer taste and so it could be more attractive for the users of dental hygiene products.

EVIDENCE OF A NOVEL LINEAGE OF PUTATIVE METHANOGENIC *ARCHAEA* WITH PHYLOGENETIC AFFILIATION TO *THERMOPLASMATALES* IN HUMAN SUBGINGIVAL PLAQUE ASSOCIATED WITH PERIODONTAL DISEASE

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Human-adapted archaea have recently attracted novel attention due to increasing evidence of their (at least indirect) involvement in health and disease. With few exceptions (e.g. haloaphilic *Archaea* in the gut system) all reported phylotypes are methanogens which thrive in various anaerobic niches in the oral cavity, the vagina and the large intestine. Recently, a hitherto novel clade of *Archaea* with a common line of descent with members of the *Thermoplasmatales* has been described in the human gut system with evidence that they reflect previously unknown methanogens. This finding is surprising given that the natural environments of cultivated members of the *Thermoplasmatales* are extremely hostile. For instance, members of the genera *Picrophilus* and *Thermoplasma* can grow at the lowest pH values known among all organisms and simultaneously at high temperatures.
Purpose: Here, we test the hypothesis that *Thermoplasmatales*-related organisms distinct to those found in the human gut system may exist in the human oral cavity. Assuming a methanogenic (thus anaerobic) metabolism the subgingival plaque biofilm was conceived as the most feasible oral habitat.
Methods: Our analysis encompassed subgingival plaque samples from 30 German adults at different stages of severity of chronic periodontal disease (the most common form of periodontitis). For selective gene amplification, a set of different primer pairs with intended target-specificity to the novel *Thermoplasmatales*-clade was employed. Besides the 16S rRNA gene we analyzed also the *mcrA* gene, encoding for an enzymatic key step in methanogenesis.
Results: Three individuals (two females, aged 67 and 89, and one male, aged 69) were tested positive for both genes. Generation of clone libraries and sequence analysis revealed unique 16S rRNA gene types within the phylogenetic radiation of *Thermoplasmatales*. In addition, the *mcrA* gene type formed a novel lineage moderately related to the human gut sequences and only distantly related to known methanogenic species (approximately 67% identity at the level of amino acid sequence to *Methanopyrus kandleri*).
Conclusion: Uniqueness and high identity of the sequence types in all positive cases argues against transient but rather resident novel *Archaea* possibly specifically adapted to the human oral cavity. Concurrence of 16S rRNA gene and *mcrA* gene types suggests that this novel lineage corresponds to a hitherto unknown group of methanogens.

PI-19

THE RISK FOR DEVELOPING ORAL INFLAMMATION UNDER STRESS IS POSSIBLY PREDICTABLE BY ANALYSING THE PREFORMED ORAL ANAEROBIC FLORA

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The bacterial inventory of the human oral cavity is already fairly comprehensive and composed of about 800 different species many of which are uncultivated. However, the dynamics and variability of the oral microflora in relation to health or disease are still poorly understood.
Purpose: Our aim was to test the hypothesis that the development of oral symptoms after multiple stressing of the immunosystem is related to the composition and diversity of the initial microflora.
Methods: As a model, we analyzed the oral microbiota from 58 healthy individuals during a challenging (hygiene, temperature, physical and mental stress) expedition in remote regions of the Himalayans (Anapurna). Plaque samples were taken at begin (Bhulbule) and destination (3,000 meter difference in altitude) seven days later (Manang). Twenty-eight individuals were symptom-free (Group I) while 30 participants developed dental problems, mostly gingivitis (Group II). The oral microbiota was monitored via terminal restriction fragment length polymorphism (T-RFLP) analysis of amplified 16S rRNA-genes directly from the plaque samples. Variation in T-RFLP datasets were analyzed based on the Additive Main Effects and Multiplicative Interactions (AMMI) model using the T-Rex software package (http://trex.biohpc.org/).
Results: Variation from T-RF main effects was at least 95% indicating that the majority of variation in T-RFLP profiles was due to inherent differences in microbial communities among individuals. However, when comparing Group I and II an interaction signal of about 3% was consistently observed (under varying analytical parameters) while this was not the case when the two time points of sampling (i.e. taken at start and end of the trekking-tour) were compared. Likewise the Shannon-Weaver-index of diversity and the Jaccard-index of similarity indicated, that Group I and II differed significantly in the heterogeneity of their oral microbial communities at begin of the trekking tour. Based on the Human Oral Microbiome Database (HOMD) distinct bacterial taxa (Prevotella, Fusobacterium, Neisseria) could be elucidated as key-components explaining the observed differences.
Conclusion: The data indicate that the initial composition of the oral ecosystem determines whether or not oral inflammatory symptoms develop upon exposure to various stress-parameters.

PERIODONTAL BACTERIA IN PREGNANT AND THEIR DISSEMINATION TO FETAL ANNEXES

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The presence of periodontal bacteria in extra-oral sites, such as vagina, amnion, and placental parenchyma, and their possible role in the poor perinatal outcome has been reported. Subgingival and obstetric (vagina, amnion and placental parenchyma) samples from 93 pregnant visiting a public hospital (Vitoria, ES, Brazil) were obtained. *Porphyromonas gingivalis* (*Pg*), *Aggregatibacter actinomycetemcomitans* (*Aa*), *Fusobacterium nucleatum* (*Fn*), and *Tannerella forsythia* (*Tf*) were identified by PCR using species-specific primers. Periodontal bacteria were detected at least in one of the four collected sites from 70 (75.3%) positive patients. In subgingival samples were detected: *Aa* in 2 (2.8%), *Fn* in 16 (22.8%), *Pg* in 30 (42.8%), and *Tf* in 29 (41.4%) patients; in vagina samples: *Aa* in 3 (4.3%), *Fn* in 2 (2.8%), *Pg* in 16 (22.8%), and *Tf* in 3 (4.2%) patients; in amnion samples: *Fn* in 4 (5.7%) and *Pg* in 9 (12.8%) patients; in placental parenchyma samples: *Fn* in 1 (1.4%), *Pg* in 4 (5.7%) and *Tf* in 1 (1.4%). The four bacteria were found in vagina; *F. nucleatum* and *P. gingivalis* in amnion; and *F. nucleatum, P. gingivalis,* and *T. forsythia* in placental parenchyma. In addition, these results suggest that most of periodontopathogens present in vagina, amnion or placenta did not belong to the pregnant' mouth, and it is possible that these bacteria belong to the sexual partner, but this hypothesis needs further studies.

PI-21

HETEROGENEITY IN THE 40KDA OUTER MEMBRANE PROTEIN IN HUMAN ISOLATES OF *FUSOBACTERIUM NECROPHORUM* SUBSPECIES *FUNDULIFORME*

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Statement of Purpose: To determine variation in the 40KDa adhesin of human *Fusobacterium necrophorum* subsp *funduliforme* isolates.

Fusobacterium necrophorum is a rod-shaped Gram-negative obligate anaerobic bacterium. Two subspecies of *F. necrophorum* are of pathogenic importance. Subspecies *funduliforme* (FNF) is a primary causative agent of recurrent and persistent pharyngitis in young adult humans. Pharyngitis may occasionally progress to septicemia and metastatic infections (Lemierre's syndrome). Subspecies *necrophorum* is the primary etiologic agent of many economically important animal diseases such as necrotic laryngitis, foot rot and liver abscesses. Attachment of bacterial pathogens to host cell surfaces is an important virulence component essential for establishment of infection and persistence. Outer membrane proteins (OMPs) of Gram-negative bacteria have been shown to play a vital role in such attachment. The OMPs are hypothesized to actively participate in binding of *F. necrophorum* to host cells to cause clinical disease. A candidate adhesin of approximately 40 kDa was targeted in this study. Previous studies have shown that a 40KDa FomA protein of *F. nucleatum*, an oral pathogen closely related to *F. necrophorum*, binds to statherin a salivary phosphoprotein. This protein facilitates binding of *F. nucleatum* to enamel surfaces to form biofilms. Sequence information of the *fomA* gene was used to design primers to amplify a 1.0 to 1.1 kb gene from four human FNF isolates. Sequence analysis of the amplified fragments demonstrated a similarity of approximately 60% and an identity of 48-50% compared to the *fomA* gene of *F. nucleatum*. There was also a considerable heterogeneity in the gene sequence among FNF isolates. A western blot assay of the total OMPs from FNF isolates using antibody raised against recombinant 40KDa protein, revealed that the size of this protein was different in the four human clinical isolates tested.

Heterogeneity in this protein may contribute to the differences in the ability of human FNF isolates to bind to host cells, and also the differences in the patterns of clinical disease.

SUBGINGIVAL MICROBIOTA IN OBESITY PATIENTS BEFORE AND AFTER BARIATRIC SURGERY

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Obesity is considered a public health problem showing an impact in morbidity and mortality in patients with metabolic disorder. Studies have shown associations between obesity and chronic periodontitis; however, it has not been sufficiently understood. In this study, possible differences in the subgingival bacterial composition and periodontal status of obesity individuals before and after surgery (Roux-en-Y gastric bypass) during 12 months were investigated. Subgingival samples were collected in four times (before surgery, 2, 6 and 12 months after surgery). Twenty three patients with or without periodontal disease, body mass indices (BMI) ≥ 40, and indication for bariatric surgery were evaluated. Thirty no obesity subjects were also included. A complete oral clinical examination was performed. Bacterial detection by PCR for *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum,* and *Dialister pneumosintes* was performed. It was observed statistically significant differences to pocket depth ($p \leq 0.001$), BMI and % fat mass ($p \leq 0.0001$) among analyzed subjects. In fat patients the detection of *T. forsythia* (23.3%) and *A. actinomycetemcomitans* (60%) was higher than in nonfat subjects (10% and 40%, respectively). No change in subgingival microbiota before and after surgery was observed. These results suggest the benefic effects of the gastric bypass surgery in the oral health, because periodontal bacteria were decreased; however, more studies are necessary to elucidate this relationship in patients with obesity.

PI-23

IDENTIFICATION OF GENETIC DETERMINANTS OF *PORPHYROMONAS GINGIVALIS* RESPONSE TO TRICLOSAN

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Our primary objective is to identify genes involved in *Porphyromonas gingivalis* response to triclosan. Triclosan is a widely-used and potent antimicrobial that exerts a bacteriostatic effect by inhibiting fatty acid synthesis, or a bactericidal effect by interfering with the functions of multiple cytoplasmic and membrane targets. Mechanisms of bacterial resistance to triclosan include increasing the expression of important regulators or metabolic enzymes and upregulation of efflux activities. Efflux mechanisms of resistance to triclosan have been shown to result in cross-resistance to multiple antimicrobials. The development of such cross-resistance by *Porphyromonas gingivalis*, an oral anaerobic bacterium associated with periodontal disease and a spectrum of systemic effects, is a growing concern. In order to identify the gene targets and pathways that are important in *P. gingivalis* response to triclosan, we used massively parallel sequencing technology to evaluate gene frequencies of a Mariner based transposon insertion library of *P. gingivalis* (strain W83) using a strategy called Tn-seq. Changes in frequency of each mutant were determined by sequencing the regions flanking the transposon and used to identify mutants that were negatively or positively affected by exposure to sub-MIC concentrations of triclosan. Inactivation of specific genes was associated with changes in triclosan sensitivity. Mutants of individual genes involved in heme utilization and amino acid metabolism were over-represented upon incubation in the presence of triclosan, while various genes including those involved in stress response, DNA replication and repair, and amino acid metabolism were under-represented. Antibiotic resistance profiles of selected strains were evaluated using a panel of antibiotics. This study demonstrates that Tn-Seq is a useful tool to identify the genetic pathways affected by triclosan and study cross-resistance in *P. gingivalis*.

1445 POSTER SESSION I: THE GENITAL MICROBIOTA

Posters will be presented in Poster Session I
Friday, June 29 1445-1545.

PI-24

BACTERIAL COMMUNITIES IN WOMEN WITH BACTERIAL VAGINOSIS: IMPACT OF MICROBIOTA ON CLINICAL VARIABLES

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Background: Bacterial vaginosis (BV) is a common condition linked with several adverse health outcomes and is characterized by the presence of diverse anaerobic bacteria with a loss of most lactobacilli. Little is known about the relationship of individual bacteria with clinical variables used to diagnose BV.
Purpose: We sought to determine the associations between vaginal bacterial community composition and Amsel clinical criteria for BV.
Methods: Broad-range 16S rRNA gene PCR and pyrosequencing was performed on vaginal samples from 220 women with and without BV. BV was diagnosed by Amsel's criteria and confirmed by Gram stain. Query sequences were classified using a phylogenetic placement tool. Associations of the Amsel variables with the bacterial taxa were investigated using penalized linear models.
Results: Species diversity and species richness was increased in women with BV. *Leptotrichia amnionii* and *Eggerthella* sp. were the only two BV-associated bacteria that were significantly associated with each of the four clinical variables. *L. crispatus* was the only *Lactobacillus* species associated with low pH, negative Whiff test, absence of clue cells and a normal vaginal discharge. *Gardnerella vaginalis* and *Atopobium. vaginae* were each correlated with 3 clinical criteria; *G. vaginalis* was not associated with an abnormal vaginal discharge and *A. vaginae* was not associated with an amine odor. Several bacteria including *Prevotella* spp., bacterial vaginosis associated bacterium 1, and *Dialister micraerophilus* were correlated with an amine odor. Edge-PCA suggested that women with high levels of *L. crispatus* have low pH and are BV negative; women with high *L. iners* levels can have either low or high pH, and many with high pH have BV.
Conclusions: Women with BV have heterogeneous vaginal bacterial communities. Different bacterial species are associated with different elements of the clinical criteria used in the diagnosis of BV, which may account for discrepancies between Amsel and Nugent (Gram stain) diagnostic criteria.

SUSCEPTIBILITY OF VAGINAL AND RECTAL *LACTOBACILLUS* SPECIES TO ANTIMICROBIAL AGENTS

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Purpose: Women reporting antibiotic use have decreased colonization by lactobacilli. The objective of this study was to describe the species-specific susceptibility of vaginal and rectal *Lactobacillus* to commonly used antibiotics.
Methods: Vaginal and rectal lactobacilli were identified to the species level using a combination of rep-PCR, 16S rDNA sequence, and BLAST. A total of 150 isolates, including *L. crispatus*, *L. jensenii*, *L. gasseri*, and *L. iners* (30 isolates each), 9 *L. vaginalis*, 9 *L. coleohominis*, 3 *L. fermentum*, 3 *L. ruminis*, 3 *L. rhamnosus*, and 3 *L. johnsonii* were tested for susceptibility to ampicillin, azithromycin, cefazolin, clindamycin, and doxycycline. The Clinical and Laboratory Standards Institute (CLSI) guidelines for anaerobic agar dilution method were used to determine the minimal inhibitory concentrations (MICs). For azithromycin, only the MIC range was described.
Results: Although all lactobacilli tested were susceptible to cefazolin, there was considerable species-specific heterogeneity in susceptibility to ampicillin, doxycycline, and clindamycin. Of 150 isolates, 25 (17%) were resistant to ampicillin; *L. crispatus* (20%), *L. jensenii* (13%), *L. iners* (10%), *L. vaginalis* (11%), *L. coleohominis* (33%), *L. rhamnosus* (67%), *L. ruminis* (100%), and *L. johnsonii* (100%). Clindamycin resistance was detected in 30 (20%) isolates, ranging from 87% of *L. gasseri* to 13% of *L. iners*. For doxycycline, 25 (17%) isolates of lactobacilli were resistant: 47% of *L. iners*, 33% of *L. jensenii*, and 33% of *L. johnsonii*. For azithromycin, there were 6 isolates having an MIC of >256 µg/mL (5 *L. iners* and 1 *L. rhamnosus*).
Conclusion: Only 20 of the 60 isolates of *L. crispatus* and *L. jensenii*, the predominant vaginal *Lactobacillus* species in normal women, were resistant to one or more commonly used antibiotics. By contrast, most (26 of 30) isolates of *L. iners* were resistant to one or more antibiotics, and most *L. gasseri* were resistant to clindamycin. Antibiotic usage may differentially impact colonization by lactobacilli.

PI-26

THE PREVALENACE OF ACTINOMYCES SPECIES IN THE VAGINAL FLORA

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Objective: The genus *Actinomyces* consists of a heterogeneous group of gram-positive, facultatively anaerobic or microaerophilic rods which are members of vaginal flora. The objective of this study was to describe the species of *Actinomyces* present in pregnant women, stratified for Nugent Gram stain pattern.
Methods: Vaginal swabs were collected from 613 pregnant women and inoculated onto agar media for detection of aerobic and anaerobic microorganisms. Purified DNA from each isolate was used for16S rRNA direct PCR and sequencing. Restriction fragment length polymorphism (RLFP) was performed using *Hae* III restriction enzyme. A library of *Hae* III restriction enzyme patterns of known species of *Actinomyces* was used to identify the isolates. Vaginal smears were Gram-stained and assessed by the Nugent criteria. Chi square for trend was calculated across the three different categories of vaginal flora: normal (NL=376), intermediate (INT=110), and bacterial vaginosis (BV=127).
Results: 230 isolates of *Actinomyces* species were recovered from 159 of the women, with 26% of women having one or more *Actinomyces* recovered by culture. The most common species of *Actinomyces* were *A. neuii* (n=100), *A. hongkongensis* (n=41), *A. turicensis* (n=35) and *A. urogenitalis* (n=24). The remaining 14% of *Actinomyces* species were *A. odontolyticus, A. radingae, A. europaeus, A. graevenitzii, A. meyeri* and *A. viscosus*. Compared to women with normal or intermediate vaginal flora, women with BV were significantly more likely to be colonized with *A neuii* ($p<0.001$), *A hongkongensis* ($p<0.001$), *A. turicensis* ($p<0.002$) or *A. urogenitalis* ($p<0.006$).
Conclusion: Multiple species of Actinomyces are associated with bacterial vaginosis among pregnant women.

PREVALENCE OF MEGASPHAERA-LIKE BACTERIA IN VAGINAL FLORA OF PREGNANT WOMEN

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Objective: Megasphaera-like bacteria phylotypes I and II are associated with bacterial vaginosis (BV) and thought to be detected only with culture-independent methods. The purpose of this study was to characterize these organisms when cultivated from vaginal specimens.
Methods: Vaginal swabs collected from 805 pregnant women were processed for quantitative cultures for facultative and anaerobic bacteria. Colonies detected on Brucella agar supplemented with 5% sheep blood grown for 4-7 days at 36°C anaerobically were subcultured and DNA was extracted. A combination of 16S rRNA gene sequencing and restriction fragment length polymorphism (RFLP) was used for identification. A vaginal smear was Gram stained and assessed according to the Nugent criteria. A chi square test for trend was calculated across the three strata of vaginal flora, normal (NL=484), intermediate (INT=149), bacterial vaginosis (BV=171).
Results: 108 isolates of Megasphaera-like bacteria were recovered from 805 women, with phylotype I being more common [81 (10%)] than phylotype II [27 (3%)]. Compared to women with NL and INT flora, women with BV were significantly more likely to be colonized with phylotype I [NL=8(2%), INT=16(11%), BV=56(33%), P<0.001] or phylotype II [NL=4(1%), INT=8(5%), BV=15(9%), P<0.001]. The mean log concentration of isolates per woman were significantly higher for phylotypes I and II in women with BV (type I=6.2, type II= 5.9) compared to INT (type I=5.2, type II= 5.7) and NL (type I= 3.9, type II=3.2), P<0.001.
Conclusion: Megasphaera-like phylotype I is more common than II, but both are associated with BV. Published studies using culture-independent methods report a higher prevalence of this novel organism compared to the culture methods used in this study (76% vs. 13%).

Anaerobe 2012

1445 STUDENT PRESENTATIONS

Judging for Student Presentations is
Friday, June 29 1145-1315.

Posters will be presented in Poster Session I
Friday, June 29 1445-1545.

SP-1

ENTRAPMENT OF SINGLE CELL ANAEROBIC MICROORGANISMS IN ALGINATE MICROBEADS

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Among all microorganisms in a soil sample (or any other ecosystem) only a small fraction can be cultivated. Anaerobic microorganisms have in general been less studied than the aerobes and that is also the case when it comes to the "un-culturable" organisms. Hence new strategies for their cultivation are needed. In order to increase the cultivation efficiency, entrapment of single cells in polymer beads and subsequent culturing to form microcolonies have been a successful strategy among different groups of aerobic microorganisms that are slow growers and difficult to cultivate using traditional techniques.

The aim of the present work was to optimize the conditions for the entrapment of single cells of anaerobic bacteria in alginate microbeads using the internal gelation system (IGS) method for further micro cultivation. For that purpose a model system was studied.

Exponentially grown bacteria from an anaerobic consortium were entrapped under anaerobic conditions in alginate microbeads. In order to optimize the IGS the oil type, presence of baffles in the beaker and the speed of the rotation impeller were varied. The formed alginate micro beads were characterized by shape and size and the results were analysed by means of ANOVA.

ANOVA analysis showed that oil type used combined with the rotation impeller speed are the most significant factors for formation of regular shaped beads with a size range of 20-80 µm. The presence of baffles can favour the formation of smaller beads, when it is required. SEM images showed spherical alginate micro beads with porous internal gel matrix that can allow the growth of the entrapped cells. Phase contrast images confirmed the entrapment of single cells. Single anaerobic cells were thus entrapped with the conditions selected and microcolony formation was successfully observed after their cultivation.

The results indicate that is possible to use alginate microbeads for enrichment of anaerobic cells which can be further used for isolation. Flow cytometry will be used in the future for sorting the microbeads containing microcolonies.

ACTIVATION OF THYMOL-β-D-GLUCOPYRANOSIDE BY BACTERIAL-EXPRESSED β-GLYCOSIDASE

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Campylobacter are a leading cause of bacterial derived foodborne illness. Thymol is a natural product that markedly reduces survivability of *Campylobacter in vitro*. Results from *in vivo* studies, however, indicate that absorption or degradation within the stomach and small intestine may preclude delivery of this compound to the cecum and large intestine, the main sites of *Campylobacter* colonization. Presently, we compared the anti-*Campylobacter* activity of thymol against that of thymol-β-D-glucopyranoside (β-thymol), the latter suspected to be resistant to degradation and absorption in the proximal alimentary tract lacking β-glycosidase activity. *Campylobacter jejuni* was grown at 39°C under N_2 in pure culture and co-culture with a β-glycosidase expressing *Bacteroides distasonis* in Bolton broth treated with or without 1 mM thymol or β-thymol. Cultures were incubated in triplicate and *C. jejuni* were enumerated by viable cell count on Campy Cefex agar. Numbers of *C. jejuni* did not differ ($P > 0.05$) between pure cultures receiving no treatment or treated with β-thymol, increasing from 7.0 ± 0.03 $\log_{10}$ CFU/mL at the start of incubation to 8.5 ± 0.08 and 8.2 ± 0.27 $\log_{10}$ CFU/mL by 48 h, respectively. Conversely, *C. jejuni* numbers in thymol-treated cultures were decreased ($P < 0.05$) by 48 h (3.7 ± 1.34 $\log_{10}$ CFU/mL) thus confirming the bactericidal activity of free thymol. Numbers of *C. jejuni* recovered after 48 h co-culture with *B. distasonis* were likewise decreased ($P < 0.05$) in thymol-treated cultures (1.3 ± 0.10 $\log_{10}$ CFU/mL) as well as in cultures treated with β-thymol (4.4 ± 0.06 $\log_{10}$ CFU/mL) thus implicating the hydrolysis of β-thymol by *B. distasonis*-expressed β-glycosidase. As expected, numbers of *C. jejuni* in untreated co-cultures (8.2 ± 0.13 $\log_{10}$ CFU/mL) did not differ ($P > 0.05$) between those measured in untreated pure cultures. These results indicate that β-thymol may be a suitable candidate to escape absorption and degradation within the proximal alimentary tract yet may be activated by bacterial-expressed β-glycosidase in the distal gut.

SP-3

IRON UPTAKE SYSTEMS IN *CLOSTRIDIUM DIFFICILE*

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Clostridium difficile, a Gram-positive, anaerobic, spore-former is the major cause of antibiotic associated diarrhoea and is also associated with more severe, sometimes life threatening disease. To date, little research has been carried out on iron uptake mechanisms and their regulation in *C.difficile*. In common with other pathogens, iron is likely to be an essential growth factor necessary for the survival of the organism and analysis of available genome sequences reveals the presence of several potential iron uptake systems and regulators. We have focussed studies on homologues of the *E. coli* ferrous iron uptake system FeoB and the ferric uptake regulator Fur. The ClosTron mutagenesis system developed in Nottingham has been used to generate knockout mutants in the three annotated *feoB* homologues and in the single *fur* homologue in *C.difficile*630Δerm. Comparisons of the growth characteristics suggest FeoB1 but not FeoB3 is involved in ferrous iron uptake.

Co-culture experiments showed that wild type out-competed the FeoB1 mutant in the presence of 2,2,-dipyridyl. Cytotoxicity assays showed that levels of the two major toxins TcdA and B were markedly reduced in the *feoB1* mutant grown under iron limitation. *Fur* mutant exhibited clear growth phenotype and was hypersensitive to hydrogen peroxide. Cytotoxicity assays and Western Blot analysis revealed decreased levels of toxins TcdA and B in the *fur* mutant. The *fur* mutant showed delayed sporulation and produced less spores than wild type. In addition, the *fur* mutant was more motile in early exponential phase than wild type and showed increased cellular iron content. RNA Seq analysis was carried out in order to investigate the *fur r*egulon in *C. difficile* and contribution to our experimental data. These findings further highlight the role of Fur and FeoB systems in regulating key and diverse aspects of physiology and virulence of *C. difficile*.

ANAEROBES ON THE HALF SHELL—ISOLATION AND IDENTIFICATION OF CELLULOLYTIC ANAEROBES FROM THE GUT MICROBIOTA OF PACIFIC OYSTERS

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The purpose of this project is to isolate and characterize naturally occurring cellulolytic, anaerobic bacteria by phenotypic, and genotypic methods. We hypothesized that the gut microbiota of Pacific oysters (*Crassostrea gigas*) would be enriched for bacteria capable of digesting the cellulose in the cell wall of their primary food source, algae. 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. Cellulolytic activity was quantified by measuring the clearance zone in soluble cellulose agarose. Genomic DNA was isolated from organisms of interest and the 16S rDNA was amplified by PCR, sequenced, and analyzed using comparative phylogenetic methods. From our analysis of the oyster microbiota, we identified five potentially novel organisms representing *Clostridium spp.*, *Lactococcus spp.*, and *Lactobacillus spp.* that are capable of anaerobic growth and cellulose digestion.

SP-5

PHYLOGENY OF *CLOSTRIDIUM PERFRINGENS* ISOLATES ASSOCIATED WITH AVIAN NECROTIC ENTERITIS BASED ON MICROARRAY COMPARATIVE GENOMIC HYBRIDIZATION

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Clostridium perfringens type A causes poultry necrotic enteritis (NE), an enteric disease of considerable economic importance. A novel toxin, NetB, is critical to NE pathogenesis and closely associated with virulent strains. We recently demonstrated that *netB* resides on a large, conjugative plasmid within a 42 kb pathogenicity locus (NELoc-1) that, in conjunction with two other loci (NELoc-2 and 3), is highly conserved in virulent strains. Though plasmid-encoded genes are clearly important to NE pathogenesis, it is not known whether a specific chromosomal background is associated with virulence.

To study further the genomic signature of NE-associated strains, we used a *C. perfringens* microarray based on two NE-producing strains to analyse the gene content of a set of 54 *C. perfringens* isolates from diseased and healthy birds by comparative genomic hybridization (CGH). A total of 128 genes significantly associated with *netB* were identified, including genes related to iron-acquisition and carbohydrate utilization.

Phylogenetic analysis of CGH data placed more than 90% of the poultry isolates into three major clusters (I-III). Group I (n=17) consisted entirely of *netB*-negative isolates, while Groups II (n=18) and III (n=15) were comprised of 61% and 67% *netB*-positive isolates, respectively. Several chromosomal loci were identified that differentiate Groups II and III, including genes for dTDP-L-rhamnose biosynthesis, D-glucuronate catabolism and capsular polysaccharide.

These findings suggest that specific chromosomal loci are predominantly associated with *netB*-positive strains, consisting of supplementary fitness-related genes that likely enhance virulence. Furthermore, this chromosomal background can be further divided into two main lineages that are predicted to differ in terms of carbon utilization and serotype.

THE *recA* OPERON: A NOVEL STRESS RESPONSE GENE CLUSTER IN *BACTEROIDES FRAGILIS*

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Bacteroides fragilis is a human gut commensal and an opportunistic pathogen causing anaerobic abscesses and bacteraemias which are treated with metronidazole, a DNA damaging agent. This study investigated the responses of the *B. fragilis recA* gene cluster to the stress experienced by the bacteria during metronidazole treatment and exposure to the reactive oxygen species generated by the host immune system during infection. Physiological studies showed that cell survival following metronidazole and hydrogen peroxide exposure was decreased in recombinase negative *B. fragilis recA* mutants, while overexpression of the RecA protein in *B. fragilis* resulted in increased survival of cells exposed to metronidazole. RT-PCR of *B. fragilis* cDNA showed that the *recA* gene was co-transcribed as an operon together with two upstream genes which are possibly involved in repairing oxygen damage. Quantitative RT-PCR was done using RNA extracted from untreated cells as well as after metronidazole and hydrogen peroxide treatment. A transcriptionally regulated response to both sets of treatment conditions was seen, with all three genes being up-regulated as a group as well as individually in response to these stress conditions. *In vivo* analysis of the upstream genes by heterologous complementation and protein expression (in *E. coli*), with subsequent biochemical assay, showed that the 2nd gene in this operon is a functional bactoferritin co-migratory protein (BCP), a small thiol-specific protein with antioxidant properties. This up-regulation and *in vivo* characterisation supports the hypothesis that the genes of the operon are involved in protection of the bacteria from the oxidative burst during tissue invasion and may play a significant role in bacterial survival and metronidazole resistance during treatment of *B. fragilis* infections.

SP-7

EXTRACTS OF NATIVE PLANTS FROM ARGENTINA AND ENTERIC CLOSTRIDIAL DISEASES

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In order to find new active compounds against *Clostridium perfringens* and *C. difficile* and their toxins, several plant extracts of the native florae of Argentina were tested.

A microdilution test to screen 100 plant extracts against *C. perfringens* and cultures in agar plates to test them against *C. difficile* were used. Minimal inhibitory concentration was calculated and the most active extracts were analyzed to see if they could inhibit the biological and cytotoxic activity of alpha and epsilon toxin. Also, the effect over alpha toxin mRNA was studied by RT-PCR. Finally, we assessed the ability of some extracts in reducing the number of bacteria in broiler chicken gut.

We found 18 active extracts (MIC below 250 µg/ml) against *C. perfringens* and 2 against *C. difficile*. We found that the 3 of these extracts were able to inhibit the alpha toxin activity from 59% up to 100%, and 1 could inhibit the cytotoxicity of epsilon toxin from 46% to 76%, depending on the pre-incubation time of the epsilon toxin and the extract. These extracts showed bactericidal activity although were not able to modified the synthesis of alpha toxin mRNA. In an animal model, one of these extracts reduced the incidence of enteritis as well as the number of *C. perfringens* in the feces.

EXPERIMENTAL STUDIES OF PROBIOTIC TRAITS OF WILD ORAL LACTOBACILLI

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The most abundantly used probiotic strains come from the genus *Lactobacillus* and only a few studies have investigated their role in oral cavity. Generally recognized as safe (GRAS), lactobacilli could play a beneficial role by inhibiting the growth of some oral pathogenic bacteria justifying their use for oral health preservation. To establish the potential health benefit of probiotic candidates, appropriate *in vitro* tests are required.

The aim of this study was to investigate the probiotics traits and properties of salivary lactobacilli that could be used for oral health. Seventy strains isolated from saliva and identified by the PCR method were screened.

First, we evaluated the adherence of growing cells on a glass surface. Adherence was scored from 0 (no adherence) to 4 (firmly adherent). All determinations were performed in triplicate.To verify the involvement of exopolysaccharides in this type of adherence, PCR with degenerated primers were performed in order to highlight the presence of glycosyltransferases (GTF).

Antibacterial activity against some oral bacterial pathogens implicated in dental decay (*S. mutans*, *A. viscosus*) or in periodontal diseases (*F. nucleatum, P. gingivalis*) was assessed by an agar overlay technique.
In order to investigate the mechanism of action of the antimicrobial effect, we tested the effect of various concentrations of lactic acid on the indicator strains. A second treatment with protease was used to reveal if the origin of the antibacterial effect was a protein. Each experiment was repeated three times.

A rapid screening of the glycerol dehydratase (GD) gene using the PCR method was also made to search possible implication of reuterin as an antimicrobial compound. Among the 70 strains, 13 were scored 3 or 4, and 57 did not form any biofilm on glass surfaces.

Our results show several putative genes for GTF in the most adherent strains on glass tube. Many strains show antibacterial properties but only one is positive for the GD gene. These initial results are encouraging in the identification of one or more strains with probiotic properties useful for oral health.

SP-9

CHARACTERIZATION OF A MarR FAMILY REGULATOR IN *BACTEROIDES FRAGILIS* RESISTANCE TO OXIDATIVE STRESS

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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 determinants such as capsular polysaccharide complex. Aerotolerance seems to contribute decisively in the process of interaction with the host and establishment of the infection. 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. Members of the MarR family have been found in *B. fragilis* strain 638R and the aim of this study is to evaluate the role of one of these regulators, BomR (BF0574), in the species survival in the host. An insertional mutation was performed in the gene coding for the BomR protein. The mutations was complemented with the BomR gene in the plasmid pFD1045 under p-OSU promoter control. Inhibition of growth by oxygen in a soft agar tube and colony counts after exposure to O_2 were used to evaluate changes in response to oxidative stress caused by the mutation. Results showed that inactivation of BomR turned the strain more susceptible to oxygen exposure than the parental strain. Reconstitution of the gene expression partially restored the aerotolerance phenotype. 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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845 POSTER SESSION II: CLOSTRICIUM DIFFICLE

Posters will be presented in Poster Session II
Saturday, June 30 845-945.

PII-1

ANALYSIS OF PerR IN OXIDATIVE STRESS RESPONSE OF *CLOSTRIDIUM DIFFICILE*

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Clostridium difficile, a spore-forming anaerobic bacillus, is identified as a major cause of nosocomial infectious diarrhea. Over the past few years, the incidence of *C. difficile* associated disease (CDAD) outcomes has increased causing a high morbidity and motility due to the emergence of hypervirulent strains and changes in the antibiotic usage patterns. While *C. difficile* colonizes in the intestine, many stresses such as oxidative stress could be involved. A recent study revealed the effect of oxidative stress on the transcriptional gene expression in *C. difficile*. Among the putative regulators identified in *C.difficile* genome, the peroxide repressor PerR has been annotated as a putative regulator of the oxidative stress response. The function of this regulator has not yet been determined in *C. difficile*, but in *Clostridium acetobutylicum*, it acts as a repressor of genes involved in the oxidative stress response. To analyze the function of PerR in the oxidative stress response of *C. difficile*, the PerR mutant was constructed using ClosTron system. The insertional inactivation of PerR gene has been successfully generated. In this study, the growth patterns and the protein expression of PerR mutant was compared to the wild type *C. difficile* and showed no differences under anaerobic condition. However, *C. difficile* survived for short periods of oxygen exposure. Analysis of protein expression of the PerR mutant also revealed a downregulation of ~21kDa and ~98 kDa proteins after exposure to air compared to wild type strains. Following peroxide exposure, both wild type and the PerR mutant were insensitive to 300µM concentration of peroxide. However, the survival rate of both strains was decreased after exposure to concentrations above 300µM H_2O_2.

ANALYSIS OF SYMPTOMATIC PATIENT STOOL SAMPLES FOR THE CO-OCCURRENCE OF NON-IDENTICAL *CLOSTRIDIUM* DIFFICILE RIBOTYPES

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Infections that contain multiple genotypes of the same pathogen (i.e. multiple strain infections) can significantly influence the progression and outcome of disease. *Clostridium difficile* infection (CDI) ranges in severity and clinical presentation from a mild, self-limiting diarrhea to fulminant colitis and death. Currently, it is unclear how many symptomatic CDI cases are attributable to a single infecting strain (genotype). We utilized a high-throughput PCR ribotyping assay to estimate the prevalence of ribotype mixtures across the clinical spectrum of CDI at the University of Michigan Hospital and Health Systems. 54 stool samples were collected over two years (March 2010 – Feb 2012) from symptomatic patients that ranged in age from <1 year to 96 years old (median=61). Equal numbers of males and females were represented. 95 single-colonies were isolated on selective media for each stool sample. An average of 91/95 (96%) ribotyping reactions were successful per sample, yielding 4,914 comparable results. The prevalence of multiple strain infections was 13.0% (7/54). Mixed CDI infections were not restricted to the setting of CDI onset as defined by recommended surveillance definitions (Hospital Onset-HealthCare Facility Associated, Community Onset-HealthCare Facility Associated, Indeterminate, and Community Associate). Nor did they occur more often among older individuals. Mixed CDI infections did not appear to be associated with any particular ribotypes, although more cases are needed to test this hypothesis. In conclusion, a significant proportion of CDI cases are caused by two or more genotypes of *C. difficile*. This suggests that epidemiologic investigations and surveillance studies seeking to associate pathogen genotype with patient outcome should incorporate data from multiple isolates per patient stool sample.

PII-3

RIBOTYPE ABUNDANCE AND *CLOSTRIDIUM DIFFICILE* PATHOGENICITY

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Aim: To rank *C. difficile* ribotypes in feces by frequency and explain that hierarchy.
Methods and results: We ribotyped 807 *C. difficile* isolates from 3504 anonymous and unlinked samples submitted from 2009-2011. We measured the MIC_{50} of moxifloxacin (MX). Spore, toxin, GDH, and lactoferrin levels in GDH+ (n=47) and GDH- (n=13) fecal samples were assayed. Six of 63 ribotypes accounted for 64% of isolates (027 - 28%; 053 - 15%; 014 - 9%; 001, 002 and 106 – 4% each). All six were toxigenic. 027, 053, and 001 isolates had MX MIC_{50} of >32 µg/mL. Despite being non-toxigenic, the only other resistant ribotype, 039, ranked 7th and was 3% of isolates. 027 was in 29% of GDH+ and 16% of GDH- samples ($p<0.05$), a pattern reversed for non-toxigenic isolates (11% and 21%, $p<0.05$). 002 and 106, (both toxigenic) were also more abundant in GDH+ samples but not significantly so. Regardless of ribotype, there was a mean of $10^{5.97}$spore/g in GDH+ feces but only $10^{2.95}$ in GDH ($p<0.05$). Lactoferrin was also lower in GDH- samples ($p<0.05$). Fecal samples from which 027 was isolated, had more TcdB than TcdA (31 and 9 ng/mL respectively) whereas in samples yielding 053, the ratio was reversed (1 and 11 ng/mL). For all other toxigenic ribotypes the levels were ~1 and 2 ng/mL respectively.
Discussion: Its portfolio of enhanced toxin production, binary toxin production, and MX resistance made 027 the fittest ribotype. The abundance of non-toxigenic but resistant 039 showed toxins were not essential, and that MX resistance may be currently the deciding factor in the rank of common ribotypes. GDH+ samples were significantly more often 027 positive than GDH- samples; the latter were significantly more often positive for non-toxigenic isolates. Thus fecal GDH reflected the extent of colonization and fitness of the resident ribotype. Fewer spores, less toxin and lactoferrin, and an increased frequency of non-027 were features of GDH- samples. Finally, the relatively high level of tcdB in 027 samples may contribute to the severity of symptoms.

EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE* ISOLATED FROM THE CB-183,315 PHASE 2 TRIAL

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Stool specimens were collected from the *Clostridium difficile* Associated Diarrhea randomized, controlled, double-blind, multi-center Phase 2 trial comparing the efficacy of the oral lipopetide CB-183,315 (125mg or 250mg BID) to vancomycin 125mg QID (VAN).

Subjects (210) were randomized (1:1:1) from April 2010 to April 2011 in North America. *C. difficile* isolates (182) were recovered from baseline specimens from 199 MITT subjects and tested for susceptibility using CLSI agar dilution and typed by Restriction Endonuclease Analysis and Pulsed Field Gel Electrophoresis. The incidence of BI strains was 34% (62/182), of these, 63% were from Canadian centers. Overall, 26% (48/182) of strains were identified as NAP1/BI. The distribution of BI strains was similar in all 3 arms of the trial: 29% (18/62), 36% (21/59) and 38% (23/61) in the CB-183,315 (125mg or 250mg BID) and VAN groups respectively. The *in vitro* activity of CB-183,315 ($MIC_{50/90}$=0.25/0.5mg/mL) was the same against BI or non-BI strains and against isolates with elevated MICs (MIC≥2mg/mL) for metronidazole and vancomycin. Of the 178 cured subjects, 48 had a recurrence: 28% (17/61), 17% (10/58) and 36% (21/59) from the CB-183,315 (125mg or 250mg BID) or VAN group respectively. The recurrence rates for subjects infected with a BI strain were 33% (5/15), 33% (5/15) and 52% (11/21) from the CB-183,315 (125mg or 250mg BID) or VAN group respectively. The rates of cure and recurrence were 82% (51/62) and 41% (21/51) respectively for subjects infected with a BI strain compared to 93% (111/120) and 22% (24/111) for subjects infected with a non-BI strain.

BI strains were highly prevalent in this phase 2 trial. Cure rates were lower (p=0.046) and recurrence rates were higher (p=0.014) in subjects infected with a BI strain. Although we saw a difference of 19% between either of the CB-183,315 arms and VAN, recurrence rates were not statistically different in the subset of patients infected with a BI strain. The better overall recurrence rate achieved with CB-183,315 250mg BID vs. VAN (p=0.035) may offer clinical benefits.

PII-5

OUTPATIENT INFECTIOUS DISEASES CLINIC ENVIRONMENTAL CONTAMINATION WITH *CLOSTRIDIUM DIFFICILE*

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Introduction: Ambulatory care settings are largely unexplored as a source of *Clostridium difficile* (CD) contamination despite recent studies indicating that up to 50% of patients with community-acquired CD have outpatient clinic exposures. We examined the point prevalence of CD in a hospital-based outpatient HIV and infectious diseases (ID) clinic that uses 5000 ppm bleach for room disinfection.
Methods: Environmental sampling occurred after clinic hours and after routine disinfection by clinic personnel. 20 clinic rooms (8 exam rooms, 4 bathrooms, 2 waiting rooms, 2 physician work rooms, 1 phlebotomy room and 3 other rooms were sampled Rooms with positive cultures were re-cultured 7 days later, stratifying by 28 sites (10 patient chairs, 4 physician chairs, 3 computer keyboards, 1 table top, 1 exam table, 9 other high-touch surfaces). Negative controls were included and consisted of alcohol wipes handled by each sampler for 30 seconds using gloves. 70% isopropyl alcohol pads were used to sample a 1500 cm^2 area. ≤ 10 pads/ 40 ml culture were used for room cultures, 1 pad/ 5 ml broth was used for single site cultures. Cultures were performed using enrichment in cefoxitin (15.5 mg/l) cycloserine (500 mg/l) mannitol broth with 0.1% taurocholate and 0.5% lysozyme. *C. difficile* was confirmed using standard methods..
Results: 4/20 (20%) ID clinic rooms (1 exam room, 1 physician work room, 2 waiting rooms) were CD positive. 4/28 (14%) single sites were positive in 2/4 of the positive rooms. All positive sites identified were fabric-covered patient chairs.
Conclusions: CD-contaminated outpatient clinics could potentially result in acquisition among patients at risk for CD infection. Frequent bleach disinfection of high touch areas in an outpatient setting appears to be effective, but upholstered surfaces may be impossible to disinfect consistently.

A NOVEL ECOSYSTEM THERAPEUTIC FOR THE TREATMENT OF *CLOSTRIDIUM DIFFICILE* INFECTIONS

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The human gut contains a diverse bacterial community that contributes to overall health. In some individuals, dysbiosis in this community can lead to many gut diseases. In particular, frequent use of antibiotic therapies can cause a shift in beneficial gut microbes and allow pathogenic interactions to predominate. *Clostridium difficile* associated disease (CDAD) is strongly associated with prior antibiotic exposures, and is a particular problem in the hospital setting. Treatment of CDAD, particularly recurrent CDAD, is often difficult; conventional antibiotic treatment can, ironically, exacerbate the underlying dysbiosis in the gut microbiota. In contrast, microbiota replacement strategies such as fecal transplants have shown more promising results in curing disease and restoring health. However, fecal transplants carry a significant amount of risk to the patient because the exact composition of the donor stool is largely unknown and, despite extensive screening protocols, may harbour pathogenic microorganisms. This study focuses on the preparation and use of a Defined Ecosystem Therapeutic (DET) for CDAD treatment based on known bacterial isolates from healthy donor stool. Bacteria were isolated from a fresh stool sample using various culture conditions. A chemostat was also inoculated with the same sample and allowed to reach steady state for further enrichment and isolation of bacterial diversity. Bacterial isolates were identified by 16S rRNA sequencing, and antibiotic resistance profiles were determined for each strain. The composition of the final formulation was based on acceptable antibiotic resistance profiles and representative proportions of bacterial genera from the healthy human gut. The DET was administered in one dose to two patients with severe CDAD and resulted in complete and rapid resolution of disease, with no recurrence to date. 16S rRNA gene sequence profiling suggested that the ecosystem persisted in these patients for several months following the procedure.

PII-7

DISTRIBUTION OF *CLOSTRIDIUM DIFFICILE* PCR-RIBOTYPES IN NIGERIA

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Clostridium difficile which is the commonest cause of hospital-acquired diarrhoea has become a major concern in healthcare settings worldwide. In spite of a growing numbers of studies on *C. difficile* infection (CDI) in the Western countries, such studies are limited in Nigeria where information on the prevalence of CDI is lacking. Some studies have shown high rates of *C. difficile* carriage amongst hospital workers and one study reported carriage rates amongst children but there has been no study determining the epidemiological relatedness of the strains. It is important to establish the genetic relatedness of this organism in order to establish its epidemiological and public health importance in our environment as most of the literature on CDI stems from the collection and interpretation of data from the developed countries. Isolates from diarrhoeal faecal samples obtained mainly from post-operative patients between 2005 and 2008 in four local Government Areas including a teaching hospital in Lagos, Nigeria, were subjected to PCR-ribotyping at The University of Western Australia, Australia. A total of nine ribotypes of *C. difficile* were detected which were distinct and differing by one or more bands. This showed there was wide range of *C. difficile* ribotypes circulating in Lagos. The clonal difference seen among the isolates was indicative of the fact that the isolates were obtained from patients sampled from different parts of Lagos and this has demonstrated that *C. difficile* strains are diversified and varied from hospital to hospital.

PROTEOMIC ANALYSIS OF CELL WALL PROTEIN-ENRICHED FRACTIONS REVEALS DIFFERENT PATTERNS IN BRAZILIAN CLINICAL ISOLATES OF *CLOSTRIDIUM DIFFICILE*

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Clostridium difficile is a spore-forming anaerobic intestinal pathogen that is the leading cause of nosocomial antibiotic-associated diarrhea (CDI). In Brazil, a unique ribotype (133) has been frequently isolated from children and adults. The pathogenesis of CDI is attributed to two major virulence factors, TcdA and TcdB. *C. difficile* also expresses a number of key proteins, including cell wall proteins (CWPs). S-layer proteins (SLPs) are CWPs that form a paracrystalline surface array that coats the surface of the bacterium. SLPs have a role in *C. difficile* binding to the gastrointestinal tract, but their importance in virulence is still unknown. Here we describe proteomics analysis of CWP-enriched fractions obtained through glycine extraction of five *C. difficile* clinical isolates from Brazil. Firstly, 1-D gel analysis depicted striking differences among CWP bands from some isolates. Prominent bands were cut, digested, and analyzed by mass spectrometry (MS). Identified CWPs presented different amino acid coverage suggesting differences in protein sequences. Secondly, the extracts were digested with trypsin, the peptides were separated by liquid chromatography, and introduced into the mass spectrometer (MS). Gel-independent MS analysis of the extracts confirmed CWPs as the most abundant components, but a number of other proteins could be detected as well. Moreover, we were able to identify with high confidence ~120 proteins in the fractions, among them SlpA, cwp2, cwp6, cwpV, and cwp84. Proteomics analysis of CWPs with different patterns observed among the isolates analyzed provides additional information towards in depth characterization of the strains causing CDI in Brazil.

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PII-9

ANALYSIS OF WHOLE PROTEIN PROFILE OF BRAZILIAN STRAINS OF *CLOSTRIDIUM DIFFICILE* AFTER TREATMENT WITH HOSPITAL DISINFECTANTS

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Clostridium difficile is an important enteric pathogen and it is the etiological agent of *C. difficile*-associated diarrhea. The hospital environment is one of the most important reservoirs of this microorganism, which contributes to its spread. The appropriate disinfection of some hospital areas, using the appropriate disinfectants, is essential to prevent the spread of this pathogen. The aim of this study was to analyze the whole proteins profile of different *C. difficile* strains against hospital cleaning agents usually used in Brazilian hospitals, such as: Virkon®, Cloro-Rio®, Peresal®, Riohex®, and Cidex®. The strains selected were BI/NAP1/ribotype 027; ATCC 9689; HU17 (ribotype 133) e SJ1 (ribotype 135), the last two were isolated exclusively in Brazil. Briefly, the strains were grown in the presence and absence of sub inhibitory concentrations of the specific disinfectant. A loop full of bacterial cells was treated with lysozyme and proteins were quantified by the Bradford assay. Samples were applied is a SDS-PAGE and the whole proteins of the strains grown without disinfectants revealed significative differences, which might be related to their ribotypes. Our results showed that the protein profile of BI/NAP1/ribotype 027 strain changed in the presence of all disinfectants tested. Concerning the other three strains, changes were inconsiderable when they were grown with Virkon®, Cidex® and Peresal®. On the other hand, disinfectants Cloro Rio® and Riohex® changed significantly the expression of certain proteins in all strains. In fact, Riohex® showed a great alteration of proteins profile. Taken together, our results demonstrated that some disinfectants might affect the expression of certain proteins in *C. difficile* and their study can help us to elucidate the resistance of this microorganism against some disinfectant agents and create new alternatives to eliminate/decrease this bacterium from the hospital environment.

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EFFECT OF HOSPITAL DISINFECTANTS ON SPORES OF BRAZILIAN STRAINS OF *CLOSTRIDIUM DIFFICILE*

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Patients with *Clostridium difficile*-associated diarrhea, excrete a large amount of spores in their stool, leading to contamination of the hospital environment and spread of the pathogen. This fact can be explained by the resistance of spores to many disinfectants used in hospital routine. The aim of this study was to evaluate the sporocidal activity of hospital disinfectants against spores of Brazilian *C. difficile* (HU17-ribotype 133 and SJ1-ribotype 135) strains. BI/NAP1/ribotype 027 strain was also used for comparison. The following hospital cleaning agents, usually used in Brazilian hospitals were tested: Virkon®, Cloro-Rio®, Peresal®, Riohex®, and Cidex®. Briefly, the spores were obtained by growing the strains anaerobically on BHI plates for 7 days and subsequent cell suspension in sterile water. Samples were heated at 70ºC/15 min and maintained at 4ºC. Suspensions were placed on glass carriers which were submerged in disinfectants in the concentration and during the time recommended by the manufacturers. Water was used as control of all experiments. After neutralizing the disinfectants, spores survival were determined by CFU counting after 5 days/37ºC anaerobically. The reduction of the number of viable spores was measured by comparing the Log_{10} of CFUs obtained after exposure to disinfectants with the ones obtained in control. Cloro-Rio® and Cidex® completely eliminated spores of all strains tested, while Riohex® did not show any significant reduction. On the other hand, Virkon® significantly reduced HU17 and BI/NAP1/027 spores, but the same was not observed with SJ1. Peresal® was also efficient, eliminating completely the HU17, BI/NAP1/027 and SJ1 spores. Taken together, our results show that Cloro-Rio® and Cidex® are the most remarkable agents for eliminating spores, indicating that its sporocidal activity can be chosen for routine disinfection of hospital environment in Brazil.

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PII-11

DEPRESSION, ANTIDEPRESSANT MEDICATIONS AND RISK OF *CLOSTRIDIUM DIFFICILE* INFECTION

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Previous research suggests that patients receiving antidepressant medications may have an increased risk of *Clostridium difficile* infection (CDI). We tested this hypothesis by using two databases: clinical data from adults admitted to an academic hospital (n=2,277) and population-based data of a nationally-representative sample of older Americans (n=16,781). In the clinical database, patients who tested positive for hospital-acquired CDI were compared with patients who tested negative. After adjusting for age, gender, transplantation status, antibiotics, immunosuppressants, proton pump inhibitors, and histamine-2 blockers, the odds of CDI increased 2% with each antidepressant taken prior to CDI sample collection (p=0.03). Specifically, the use of mirtazapine was most strongly related to CDI with an odds ratio of 3.29 (95% CI: 1.81, 6.01). In the population-based database, the rates of CDI in older Americans diagnosed with depression (ICD-9 codes 296.2x, 296.3x, 300.4, 311) and those without depression were 400/100,000 person-years (p-y) and 152/100,000 p-y (p<0.001), respectively. The incidence rate ratio for the relationship between ever reporting loneliness and CDI was 1.34 (95% CI: 1.13, 1.59). Marital status was also related to CDI; individuals who were divorced or separated at the time of the first interview had a CDI rate of 286/100,000 p-y and individuals widowed had a CDI rate of 322/100,000 p-y; both rates were significantly higher than individuals who were married or partnered. After adjusting for age, gender, race, body mass index, smoking, alcohol use, region of residence, chronic lung disease, stroke, end stage renal disease, inflammatory bowel disease, irritable bowel syndrome, and total number of infection-related medical visits/stays, both marital status and depression remained significantly related to CDI (RR for depression = 1.99; 95% CI: 1.59, 2.49). We conclude that depression and/or the use of certain antidepressant medications may influence the risk of developing CDI.

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SMOKERS HAVE HIGHER RATES OF *CLOSTRIDIUM DIFFICILE* INFECTION: A POPULATION-BASED STUDY

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Smoking has been shown to disrupt the microbiota of the respiratory tract, but there is little information regarding the effect of tobacco use on the composition of intestinal microbiota. Since research has shown that smokers have different diets than non-smokers, we hypothesized that smoking could potentially alter the gut environment and affect the incidence of *Clostridium difficile* infection (CDI). Therefore, we conducted a longitudinal study using a nationally-representative sample of older Americans, linking data from the Health and Retirement Study to Medicare files (n=16,781). We extracted individual-level data regarding CDI (ICD-9 code 008.45) using information from all hospital stays, skilled nursing facility stays, emergency department visits, outpatient and clinic visits, and home health visits from 1991-2007. Analyses were survey-weighted to reflect the population rates for Medicare beneficiaries across the United States. The overall rate of CDI in older Americans was 221/100,000 person-years (p-y) (95% CI: 193, 248). The respective rates of CDI for current, ever, and never smokers were as follows: 290/100,000 p-y (95% CI: 228, 353); 245/100,000 p-y (95% CI: 211, 279); and 189/100,000 p-y (95% CI: 156, 222). Rates of CDI were higher in individuals who ever smoked cigarettes compared to those who did not (p=0.01). The rates were also greater in those who were current smokers versus those who were not current smokers (p=0.02). Compared to never smokers, the incidence rate ratio for current smokers was 1.65 (95% CI: 1.26, 2.17; p=0.001) and for former smokers was 1.23 (95% CI: 1.00, 1.51; p=0.048), after adjusting for age, gender, race, ethnicity, body mass index, alcohol use, marital status, region of residence, chronic lung disease, stroke, end stage renal disease, Crohn disease, celiac disease, irritable bowel syndrome, ulcerative colitis, and total number of infection-related medical visits/stays. We conclude that smoking habits may increase the risk of CDI.

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PII-13

ROLE OF THE HUMORAL IMMUNE RESPONSE TO TOXIN B IN SUSCEPTIBILITY TO *CLOSTRIDIUM DIFFICILE* INFECTION

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Toxin B is an important virulence factor in *Clostridium difficile* infection (CDI). The objective of this study was to determine the role of antibodies to Toxin B in determining patient susceptibility to CDI.
Methods: An ELISA was used to measure total antibody levels in 38 patients (20 cases of acute CDI and 18 controls) admitted to a large teaching hospital in the UK. Baseline serum samples were collected. Plates were coated with 50µL of Toxin A or B (1µg/mL).Detection of total bound antibodies in serum was achieved using a conjugated goat antihuman IgG/A/M: HRP antibody at a dilution of 1:10,000. Pooled Intravenous immunoglobulin (Vigam Liquid®) was used as a positive control (1:1600) and to define a cut-off value. The ELISA was repeated in 40 patients admitted to the University of Michigan Hospital, (20 acute cases of CDI and 20 age-matched controls).
Results: In the UK cohort, patients had a three-fold lower anti-Toxin B titre than controls (1:18.75 IQR 1:12.5 - 1:175 vs 1:200 IQR 1:43.75 - 1:250, p=0.031). However, this was not seen for Toxin A (p=0.357).These results were replicated in the American cohort where the median anti-Toxin B titre was two-fold lower in patients than controls (1:150 IQR 1:12.5 - 1:400) vs 1:300 IQR 1:25 - 1:800, p=0.028). Again, this was not seen for Toxin A (p=0.548).
Conclusions: We have demonstrated that a pre-existing total antibody response to Toxin B is important in susceptibility to acute CDI while in hospital, in two separate cohorts. This is in contrast to previous studies that have focused on the humoral response to Toxin A and on susceptibility to relapse. These findings are in keeping with recent evidence that Toxin B rather than Toxin A may be the principal virulence factor of *C. difficile.* Measurement of immune responses to *C. difficile* could be used assess patients for susceptibility to infection and stratify treatments and infection control measures.

ISOLATION AND CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* FROM HUMAN AND ANIMAL FAECAL SAMPLES

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Introduction: *Clostridium difficile* is a major cause of nosocomial antibiotic associated diarrhoea and colitis in humans and has been found in both diarrhoeal and non-diarrhoeal animals, suggesting a potential reservoir for human infection.
Aim: Characterization of Bulgarian *Clostridium difficile* strains isolated from human and animal fecal samples.
Materials and Methods: Total of 90 faecal samples were investigated: 65 of them were from patients with mild to severe enterocolitis and previous antibiotic treatment and 25 were from healthy horses. Isolation and identification of strains was done by conventional methods. Strains were typed and further characterized for the presence of toxins A (TcdA), B (TcdB) and binary toxins (CdtA and CdtB) by PCR.
Results and Discussion: *C. difficile* was isolated from 18 of all samples. Fifteen of the strains were from human and 3 from animal origin. From the human isolates 6 (40%) belonged to PCR ribotype 017 ($TcdA^-$; $TcdB^+$; $CdtA/B^-$), followed by 002 and 014. The remaining *C. difficile* isolates from humans were grouped as 012, 046 and 078 ribotypes. All of the identified ribotypes have been reported to cause oubreaks worldwide with high lethality rate. Five of the patients from the present investigation diagnosed as *Clostridium difficile* associated diarrhoea died. The three strains isolated from horses were untypable and did not belong to any of the available ribotypes.
Conclusion: This initial investigation of *C. difficile* ribotypes among human isolates in Bulgaria has revealed that 017 (40%) is the most prevalent one. All determined ribotypes were found to be associated with severe infection and high percentage of lethal outcome. The animal *C. difficile* isolates did not belong to any of the available ribotypes.

PII-15

PATHOPHYSIOLOGY OF *C. DIFFICILE* INFECTION

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Little is known about the pathophysiology of *C. difficile* infection (CDI). Pathophysiological characteristics can be used to learn more about the mechanisms of diarrhea in CDI. The objective of the study was to compare stool characteristics between CDI-positive and CDI-negative patients with antibiotic associated diarrhea (AAD).

Fecal samples from 41 patients with antibiotic associated diarrhea from a large teaching hospital in Houston, Texas were enrolled in the study (20 CDI positive and 21 CDI negative). Fecal 1-antitrypsin (normal range 3.3-90µg/L) as a screen for protein loss, pH and osmotic gap (secretory diarrhea <80mOsm/Kg) were estimated from the 41 stool samples. In addition, markers for intestinal inflammation, fecal lactoferrin (normal value <7.5 µg/mL) and calprotection (normal value ≤ 10µg/mL) were assayed.

Variable		Patients with CDI		P-value
		Yes (N=20)	No (N=21)	
Presence of 1-antitrypsin (µg/L)	# patients >90µg/L	8 (40%)	9 (43%)	0.9216
	Means±SD	304.16±385.00	291.81±413.31	
	Median	34.93	85.09	
	Range	23.46-1061.36	35-72-1454.41	
pH	Means±SD	6.53±0.85	7.29±0.85	0.0066
	Median	6	7.5	
	Range	6-7.5	6-8.5	
Osmotic Gap (mOsm/kg)	# patients <80mOsm/Kg	15 (75%)	10 (48%)	0.0724
	Means±SD	35.57±52.44	93.94±78.55	
	Median	33.6	81.18	
	Range	-65.2-128.4	-69.2-234.40	
Lactoferrin (µg/ml)	# patients	18 (90%)	7 (33%)	0.0002
	Means±SD	53.11±50.53	6.15±39.23	
	Median	34.2	6.25	
	Range	5.80-155.20	3.2-136.3	
Calprotectin (µg/mL)	# patients	17 (85%)	5 (24%)	0.0001
	Means±SD	28.04±17.86	8.65±8.17	
	Median	26.65	6.9	
	Range	5.90-70.20	2.20-30.20	

Stools from patients with CDI were more acidic and more often contained fecal inflammatory markers than those from patients with non-CDI AAD. A subset of both forms of AAD showed evidence of possible protein losing enteropathy and secretory form of diarrhea. More studies are needed to define the pathophysiology of CDI and non-CDI AAD and to examine more completely the subset with possible gut protein loss and secretory diarrhea mechanisms.

EFFICACY AND SAFETY OF ORAL VANCOMYCIN (V) CAPSULES FOR TREATMENT OF *CLOSTRIDIUM DIFFICILE* INFECTION (CDI): RESULTS FROM TWO RANDOMIZED CLINICAL TRIALS

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Background: Although approved for the treatment of *Clostridium difficile*-associated diarrhea (CDAD) based on data using a liquid formulation, no well controlled studies have demonstrated the efficacy and safety of oral V capsules for CDI. Data from two studies are the first to offer evidence-based efficacy and safety results for the treatment of CDAD with oral V capsules.
Methods: Two Phase 3, multicenter, randomized, double-blind, double-dummy, active-controlled, parallel-group studies included subjects ≥18 with CDAD who received oral V capsules 125 mg QID for 10 days. Comparator treatments were metronidazole and tolevamer (T), an experimental toxin binder. CDAD was defined as ≥3 loose or watery bowel movements within the 24 hours preceding enrollment, and the presence of either *C. difficile* toxin A or B or pseudomembranes on endoscopy within the 72 hours preceding enrollment. Both studies consisted of a 4week post-treatment follow-up period to assess recurrence of CDAD (rCDAD). The primary endpoint was clinical success, defined as resolution of diarrhea and absence of severe abdominal pain on Day 10. Secondary endpoints included time to resolution of diarrhea (TTROD) and rCDAD. AEs were evaluated throughout the study.
Results: Clinical success rates using the Full Analysis Set (subjects who received at least one dose of V and had any post-dosing assessment) for Studies 1 and 2, respectively, were: 81.3% (109/134) and 80.8% (101/125); median TTROD (days) was 5 and 4 for all subjects, and 6 and 4 for subjects >65 years; rCDAD occurred in 23% (25/107) and 18% (18/102). Clinical success rates for T in Studies 1 and 2, respectively, were: 46.6% and 41.8% ($p<0.001$ compared to V in both studies). The most common AEs (>10%) were nausea, abdominal pain, and hypokalemia. Nephrotoxicity following V occurred in 6% of subjects >65 and 3% of subjects ≤65 years of age.
Conclusion: Oral V capsules were superior to a toxin binder comparator in two adequate and well controlled studies. Oral V 125 mg QID for 10 days is safe and effective for the treatment of CDI.

PII-17

INITIAL EXPERIENCE WITH FIDAXOMICIN IN PATIENTS WITH MULTIPLE *CLOSTRIDIUM DIFFICILE* INFECTION RECURRENCES

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Management of patients with multiple *Clostridium difficile* infection (mCDI) recurrences remains a challenging clinical problem with little evidence-based data to guide treatment. Although many patients with mCDI will respond to vancomycin in a tapering/pulsed dosed strategy, a post-vancomycin, rifaximin 'chaser' treatment, or fecal transplant/bacteriotherapy, there are a small group of patients who are refractory to these strategies. Fidaxomicin, a newly-approved agent for CDI, is a relatively narrow spectrum agent that has a superior sustained response in comparison to vancomycin in patients with primary or first recurrence CDI episodes. We hypothesized that fidaxomicin might be useful to interrupt recurrences in patients with mCDI when given as a post-vancomycin, 'chaser' treatment.

Three mCDI patients in our clinic had failed multiple attempts to interrupt these recurrences and were maintained on low-dose vancomycin until fidaxomicin became available. These patients, aged 80 (F), 32 (F), and 67 (M) years had mCDI over a period of 24, 30, and 8 months, respectively. All of them had been initially treated with metronidazole followed by vancomycin and a tapering/pulsed vancomycin treatment strategy only to 'break through' near the end of or shortly after their vancomycin taper which ended with vancomycin given every second day followed by every third day. One patient had been given a rifaximin chaser on 2 occasions and IVIG after failed attempts at vancomycin tapers and one patient had been hospitalized with severe CDI following a slow vancomycin taper over a 5 month period. All three patients were left on vancomycin 125 mg daily or every other day for a 3-6 month period when the vancomycin was stopped and a 10-day course of fidaxomicin, 200mg twice daily, was administered. Two patients have had no CDI recurrences to date (5 & 6 month follow ups) and one patient had no recurrence for 3 months, but then had a symptomatic recurrence one week after a 3-day course of levofloxacin was given for a urinary tract infection.

Although data from well-designed trials of patients with mCDI are needed, fidaxomin may be useful in breaking the cycle of multiple CDI recurrences.

MINIMUM INHIBITORY CONCENTRATION OF SWINE *CLOSTRIDIUM DIFFICILE* ISOLATES IN KOREA

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In pigs, *Clostridium difficile* can cause neonatal enteritis and can be isolated from both diseased and healthy animals. *C. difficile* shedding by pigs is of concern as a zoonotic risk because of the potential for human exposure. The objective of this study was to determine the prevalence of *C. difficile* in pigs and minimum inhibitory concentration of the isolates to 16 antimicrobial agents.

Fecal samples of 189 pigs from 11 farms were analysed. The samples were incubated anaerobically for 48h at 37°C. PCR for *tcdA* and *tcdB* was performed to distinguish toxigenic strains. To determine MICs, we performed reference agar dilution procedure using 42 isolates. *Bacteroides fragilis* ATCC 25285 and *C. difficile* 700057 were included on each plate for quality control.

In total, 42 (22.2%) *C. difficile* were isolated. The prevalence was higher in diarrhea samples at 35.8% than in non-diarrhea samples at 14.8%. Among the isolates, 35 (83.3%) were originated from suckling piglets. The prevalence, 73.3%, was the highest in diarrheic suckling piglets. Both *tcdA* and *tcdB* genes were detected in 37 isolates (88.1%). All *C. difficile* isolates tested had low MICs of ≤ 1 μg/mL for ampicillin and vancomycin, ≤ 0.5 μg/mL for metronidazole, and ≤ 0.015 μg/mL for rifaximin. However, all isolates had MICs of ≥ 64 μg/mL for ceftiofur, cefoxitin, imipenem, and bacitracin, ≥ 8 μg/mL for ciprofloxacin. Thirty-nine (91.9%) and 35 (83.3%) isolates had MIC of ≥ 8 μg/mL for enrofloxacin and clindamycin, respectively. The resistance of *C. difficile* isolates for erythromycin, chloramphenicol, and tetracycline was 31%, 2.4%, and 42.9%, resepctively. The MIC_{50} and MIC_{90} for tylosin were 0.5 and 128 μg/mL, respectively. And, those for tiamulin were 32 and 256 μg/mL, respectively. MIC values for QC strains were well within published guidelines.

Because *C. difficile* can be transmitted to human beings via the food chain, more consideration should be given when planning for the health care of pigs. In addition, these susceptibility results are encouraging us to select proper antimicrobials following accurate diagnosis for treatment of *C. difficile* infection in pigs.

PII-19

COMPARISON OF *IN VITRO* ANTIMICROBIAL SUSCEPTIBILITY OF *CLOSTRIDIUM DIFFICILE* STRAINS AS PLANKTONIC VERSUS BIOFILM STATES USING THE CALGARY BIOFILM DEVICE

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Objectives: It is hypothesized that *C. difficile* infection (CDI) may occur in a biofilm state and that antimicrobial susceptibility tests involving planktonic organisms may not be predictive of response to treatment and may play a role in persistence/recurrence. The role of biofilms in CDI was explored by cultivating *C. difficile* biofilms *in vitro* and comparing planktonic versus biofilm susceptibilities.
Methods: Eight clinical isolates (3 ribotype 001 and 5 ribotype 027 strains) and ATCC strains 43255 and 9689 were assessed for biofilm formation using the Calgary biofilm MBEC device. The device was preconditioned with hydroxyapatite for improved biofilm production (Innovotech, Edmonton, Canada). Susceptibility to 34 antibiotics was evaluated using pre-fabricated sensititre plates (TREK Diagnostic Systems, Cleveland, USA). Biofilms were cultured anaerobically in supplemented Brucella broth for 48 hours and challenged with antibiotics in fresh media for an additional 48 hours. Minimum biofilm eradication concentrations (MBEC) and planktonic minimum inhibitory concentrations (MIC) were determined. Incidence of spores in biofilms was measured after shocking with ethyl alcohol; parallel experiments were conducted with planktonic counterparts.
Results: Mean $\pm$ SD planktonic MICs (ug/ml) **/** biofilm MBEC (minimum biofilm eradication concentration) (ug/ml) results for the following antimicrobials were observed for 10 strains: vancomycin 1.6 $\pm$ 0.5 / 13.8 $\pm$ 10.4; metronidazole 0.7 $\pm$ 0.3 **/** 4.9 $\pm$ 1.4; penicillin G 2.3 $\pm$ 0.8 **/** 6.7 $\pm$ 1.0; ampicillin 1.9 $\pm$ 0.3 **/** 6.1 $\pm$ 2.3; piperacillin 6.0 $\pm$ 2.1 / 45.3 $\pm$ 17.9; daptomycin 1.8 $\pm$ 0.4 / 5.4 $\pm$ 2.9; fusidic Acid 1.3 $\pm$ 0.5 / 4.2 $\pm$ 1.9; tigecycline 0.2 $\pm$ 0.06 / 0.6 $\pm$ 0.2; rifaximin 0.02 $\pm$ 0.0 / 0.04 $\pm$ 0.03. Increased tolerance of biofilm populations towards 26 antibiotics was observed. No difference in tolerance was noted between biofilms of 001 and 027 ribotypes. Under these test conditions, sporulation was not observed in biofilm populations.
Conclusions: *C. difficile* has the ability to form a biofilm under in vitro conditions. As a biofilm *C. difficile* demonstrates increased tolerance towards treatment antibiotics, whereas some antibiotics show minimal change from planktonic values. These findings may have clinical utility.

GENOMIC CHARACTERIZATION OF A *CLOSTRIDIUM DIFFICILE* ISOLATE WITH AN ELEVATED MINIMUM INHIBITORY CONCENTRATION TO METRONIDAZOLE

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C. difficile infection is the leading cause of infectious healthcare-associated diarrhea. Metronidazole and vancomycin are currently the first-line treatments. There have been previous reports of metronidazole resistance in *C. difficile*, however the phenotype is often unstable and lost after freezing/thawing. The purpose of the current work was to characterize an isolate of *C. difficile* displaying a markedly elevated MIC to metronidazole (MIC=16 mcg/mL).

In 2009, our laboratory received a *C. difficile* clinical isolate as part of the Canadian Nosocomial Infection Surveillance Program (CNISP) which displayed a metronidazole MIC of 32 mcg/mL by E-test. The isolate's MIC diminished to 2 mcg/mL (herein referred to as "initial") after a freeze/thaw at -80°C. However, the original clinical isolate was passaged on BAK+5%LSB+ 8µg/ml metronidazole leading to growth of an isolate with a higher and stable MIC of 16 mcg/mL (herein referred to as "stable") even after multiple freeze/thaws at -80°C. PFGE analysis demonstrated indistinguishable fingerprint patterns corresponding to the NAP1 epidemic strain for both the "initial" and "stable" isolates, and both contained *tcdA*, *tcdB*, an 18-bp deletion in *tcdC,* and *cdtB* as determined by PCR. The genomes of both isolates were sequenced using 454 pyrosequencing and Illumina and underwent phenotypic analyses.

The sequencing revealed 35 single nucleotide polymorphisms or insertion/deletion mutations within gene coding sequences. These mutations included genes possibly involved in altered metronidazole susceptibility such as iron metabolism (e.g. ferric uptake regulation protein) and electron transport (e.g. pyruvate-flavodoxin oxidoreductase). Phenotypically, the stable isolate showed reduced growth in liquid BHI media (longer lag phase and entry into stationary phase that was 2 logs below the initial isolate); slower growth and smaller colony size on semi-solid media was also observed. To the best of our knowledge, this is the first study to provide an in-depth genomic and phenotypic analysis of an isolate of with a stable elevated MIC to metronidazole.

PII-21

A SURVEY OF METHODS FOR FAST *CLOSTRIDIUM DIFFICILE* TOXIN ENRICHMENT FOR PROTEOMIC ANALYSIS

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Proteomics and mass spectrometry (MS) have been extensively used in different areas as a modern approach to expedite biomarker detection and the development of sensitive qualitative and quantitative analytical protein methods. Previously, we have applied a sensitive, label-free liquid chromatography-MS method that can detect and quantify *Clostridium difficile* toxins TcdA and TcdB in small samples. We have found that sample preparation is a critical step prior to toxin detection by MS. Here, we report on the development of a fast method for toxin enrichment from *C. difficile* culture supernatants (CS) that can be directly used for proteomics studies. Analysis of samples with high toxin concentration, such as a pre-tested commercial preparation, indicated that any method (direct analysis, acetone precipitation [ppt], molecular cutoff spin filters, ammonium sulfate ppt, TCA-acetone ppt, and immunoprecipitation [IP]) could be successfully used prior to proteomics analysis. However, testing of CS from several clinical *C. difficile* isolates (NAP1, NAP2, NAP4, NAP6, NAP7) demonstrated that only TCA-acetone ppt and IP were reproducible sample preparation methods for detection and quantification of TcdA and TcdB. TCA-acetone ppt has potentially broad applicability because additional proteins can be extracted and further analyzed by MS. Using this method, the total number of CS proteins detected varied from 40 to 65 among the isolates analyzed. In contrast, the main advantage found with IP was the enrichment of TcdA and TcdB, allowing further quantification in small samples. Therefore, we used both methods to analyze CS from NAP1, NAP2, and ATCC43255 obtained after 12, 24, and 48 hours of growth. TcdA was detected in all CS within 12 hours of incubation but quantifiable amounts of TcdA and TcdB could only be determined unambiguously after 48 hours of incubation. In conclusion, both TCA-acetone ppt and IP are effective methods for sample preparation of the studied *C. difficile* CS prior to proteomics analysis.

EVALUATION OF RESISTANCE TO VANCOMYCIN AND TIGECYCLINE IN *CLOSTRIDIUM DIFFICILE*

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Clostridium difficile is an anaerobic bacterium, often associated to nosocomial diarrhea and antibiotic therapy. The most commonly antimicrobials used in the treatment of *Clostridium difficile* infection (CDI) are metronidazole and vancomycin. Despite the rare resistance to these two drugs, therapeutic failures are recurrent. The tigecycline, a new glycylglycine that presents a broad-spectrum antibacterial activity, has been suggested as a potential substitute for the classic therapy in these cases. The aim of this study was to analyse tigecycline and vancomycin susceptibility in *Clostridium difficile* strains isolated between 2000 and 2010 in Rio de Janeiro – Brazil. It was held the E-test® for the determination of minimum inhibitory concentration (MIC) to vancomycin (VAN) and tigecycline (TGC) in 23 strains, from 14 different ribotypes. Quality control followed CLSI guidelines using the American Type Culture Collection (ATCC) strains *Staphylococcus aureus* ATCC 29213. For vancomycin to which no standard breakpoint to *C. diffcile* has been defined, breakpoint was considered ≥ 8 mg/L. As expected, none of the 23 strains showed resistance to vancomycin in this study, but 6 strains showed a decreased susceptibility to this antimicrobial. Although there is no standard breakpoint for tigecycline for *C. difficile*, this drug exhibited good activity *in vitro* against the 23 isolates from Brazil. The tigecycline MIC range was ≤ 0,125 – 2,0 mg/L. Tigecycline good activity against some Brazilian isolates suggests that this drug may prove to be a complement or even a substitute to the classical therapy with vancomycin and metronidazole.

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PII-23

FIDAXOMICIN MOLECULAR MODELING AND CONSEQUENCES FOR REDUCED-SUSCEPTIBILITY MUTANTS

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Purpose. Fidaxomicin (FDX) is a novel antibiotic that was recently approved in the US for treatment of *C. difficile* associated diarrhea. FDX works via inhibition of the bacterial RNA polymerase (RNAP). Although no FDX-RNAP co-crystal structure is available, clustering of mutations linked to reduced susceptibility in the RNAP, suggests that FDX may bind near the DNA contact site. In this work, a homology model of *C. difficile* RNAP was constructed and FDX's binding mode predicted by docking and molecular dynamics simulation.
Methods. The C. *difficile* RNAP homology model was built based on the crystal structure of *Thermus thermophilus* RNAP complexed with myxopyronin. FDX was docked into the model using Autodock Vina with a 32*30*32 $Å^3$ grid box that included the mutation-containing region. Molecular dynamics (MD) simulations were performed from the representative structures of 3 top-ranked pose clusters to generate ensembles of complex structures. MM/GBSA based binding free energies were calculated to evaluate the binding affinities.
Results. The FDX binding site overlaps a portion of the RNAP switch1 and switch2 domains. Super-imposition of DNA (from the *E. coli* transcription initiation complex) into the FDX-bound RNAP, shows FDX in close proximity to the DNA melting site. Reductions in *C. difficile* mutant sensitivities to FDX can be explained by changes in hydrophobic and/or electrostatic interactions and hydrogen bonding between RNAP and FDX. Although the model demonstrates that FDX binds near the site of DNA melting, RNAP mutations are located near FDX on the opposite side of the transcription bubble.
Conclusions. Substitution of key amino acids in the *C. difficile* RNAP may directly disrupt hydrophobic interactions between mutated residues and FDX, or alternatively, may break hydrogen bonds, weaken hydrophobic interactions, or alter electrostatic interactions that would typically exist between the native and the neighboring amino acids that interact directly with FDX.

CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* STRAINS FROM PATIENTS WITH MORTALITY ATTRIBUTED TO *CLOSTRIDIUM DIFFICILE* INFECTION

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Strains of *Clostridium difficile* are associated with outbreaks and increased mortality. The purpose of this study was to characterize *C. difficile* strains based on strain identity, functionality of toxins, and clonal relatedness from patients whose death was attributed to *C. difficile* infection (CDI) determined by 2 independent investigators compared to matched controls at a university hospital in Texas. Stool samples from symptomatic CDI case patients with attributable mortality (n=12) and matched CDI control patients with mild disease who survived at least three months (n=12) were collected. *C. difficile* strains were characterized using a multiplex real-time PCR assay that detects *tcdA* (toxin A), *tcdB* (toxin B), and *cdtA / cdtB* (binary toxin) genes simultaneously. Toxin functionality was performed using a quantitative assay that measures the substrate cleavage activities of toxins A/B. A commercially available typing method utilizing semi-automated repetitive extragenic palindromic sequence-based PCR (rep-PCR) was used to study clonal relatedness defined as 90% similarity on the dendrogram. All isolates from cases and controls were positive for *tcdA* and *tcdB* and were able to produce functional toxins. Among cases, 12 of 12 (100%) isolates carried the *cdtA* and *cdtB* genes, while 6 of 12 (50%) control isolates had both genes (p=0.0047). Among 12 cases, 5 different rep-PCR patterns were identified including a single genotype with 6 clones. From the controls, 9 different rep-PCR patterns were identified including a single genotype with 3 clones (p=0.09). In conclusion, a variety of genotypes were identified in cases and controls. All clinical isolates from CDI patients produced functional toxins A/B, independent of the outcome of the infection. Infection by *C. difficile* strains with binary toxin genes showed excess rates of death.

PII-25

PREVALENCE AND EXPRESSION OF BINARY TOXIN (CDT) AMONG DIVERSE *CLOSTRIDIUM DIFFICILE* STRAINS

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The contribution of binary toxin *Clostridium difficile* transferase (CDT) to the pathogenesis of *C. difficile* infection (CDI) is still unclear, but this toxin which is unrelated to CD toxins A & B, is characteristic of the epidemic BI/027 strain, sometimes referred to as 'hypervirulent'. In order to determine the prevalence and expression of CDT among clinical strains of CD, we screened our our exhaustive collection of over 10,000 CD clinical isolates that are well-characterized by Restriction Endonuclease Analysis (REA) typing.

Representative CD isolates from all 108 REA groups were screened for the presence of binary toxin using primers for the internal fragment of *cdtB* (gene for binding component of CDT). 21% of the 108 groups (both toxigenic and non-toxigenic) in our collection were positive for CDT. Subsequently, we amplified the CDT-encoding genes from CDT-positive isolates using full length primers for *cdtA* and *cdtB* and found variations mainly in the enzymatic coding region of *cdt*A. PCR products from representative CDT-positive isolates were sequenced and aligned to published sequences. REA groups with variant CDT sequences were compared to those with non-variant sequences for differences at the transcription level using multiplex PCR (mPCR) analysis. RNA was extracted from 10 representative isolates at four different time points and mPCR was performed using short primers for *cdtA*, *cdtB*, *cdtR* and *rpoA*.

Temporal gene expression varied among strains with variant CDT sequences. Non-toxigenic groups with non-variant CDT sequences showed expression of binary toxin genes comparable to some toxigenic groups. We also compared our CDT variant data with our previous analysis of *tcdC* (gene for the negative regulator of toxins A & B in the PaLoc) and toxinotyping. Polymorphism in *tcdC* had no correlation with the presence or absence of CDT. Seven different toxinotypes had CDT in at least one corresponding REA group. Binary toxin was present and expressed in 30% (19/62) of toxigenic and 8.5% (4/46) of non-toxigenic REA groups. Further analysis of these isolates may shed insight into the role of CDT in CDI pathogenesis.

CLOSTRIDIUM DIFFICILE CARRIAGE IN A NEONATAL UNIT: EFFECTS OF STRINGENT INFECTION CONTROL MEASURES

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Purpose: To determine the carriage rate of *Clostridium difficile* in a neonatal unit and determine whether strict infection control measures may have a role in preventing colonization of newborn babies.
Methods: Stool samples from 30 neonates who were admitted immediately after birth into the neonatal unit at the Royal Infirmary of Edinburgh were taken twice weekly from the start to the end of the study (10.8.10-7.9.10). Stools were cultured on CCEY to look for *C. difficile* and isolates were characterized by PCR ribotyping. Simultaneously all other stool samples sent to the routine lab from symptomatic patients with *C. difficile* infection over the same period (total 50) were cultured and typed to determine the prevalent PCR riboytpes in the population.
Results: In total, 131 samples from babies were obtained over the four week period. Total number of samples obtained per baby ranged from 1-9. Only one stool sample (PCR ribotype 173) was positive from a baby who had been in hospital for all the four weeks of the study. This is a rare type in the prevalent symptomatic adult population (commonest being ribotypes 002, 001, 14, 20, and 15) although it was found in one adult patient in the same period as the neonatal study.
Conclusion: Similar studies have demonstrated a carriage rate of up to 62%. This study showed a 3% carriage rate. This raises the question whether better infection control practices like strict implementation of hand washing before entering a ward have contributed to the decline in *C. difficile* acquisition in the neonatal unit of the hospital. Implications of early carriage to the immunological development of neonates should be an area for further study.

PII-27

FUNCTIONAL ROLES OF THE GUT MICROBIOTA IN COLONIZATION RESISTANCE AGAINST *CLOSTRIDIUM DIFFICILE*

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Background: Antibiotics disrupt the indigenous gut microbiota, reducing intrinsic resistance to *C. difficile* colonization. The colonic microbiota plays a major functional role in supporting colonic health by fermenting complex carbohydrates and amino acids into short chain fatty acids (SCFAs) and other metabolites. Butyrate and other SCFAs provide nutrients for the gut epithelium. There is also evidence that these compounds can inhibit growth and decrease toxin production of *C. difficile in vitro.* We sought to determine if the production of SCFAs contributed to colonization resistance against *C. difficile*.
Methods: C57BL/6 mice were treated with broad-spectrum antibiotics and tested for susceptibility to *C. difficile* infection (CDI). Cecal contents from mice that were susceptible and resistant to *C. difficile* colonization were analyzed by mass spectrometry to identify the levels of SCFAs.
Results: Mice that did not receive antibiotics were completely resistant to *C. difficile* infection and had a high level of SCFAs (acetate, propionate and butyrate). Cecal SCFA levels were depressed in mice treated with either a five antibiotic cocktail ("5Abx"; gentamicin, kanamycin, colistin, metronidazole and vancomycin), 5Abx plus clindamycin or cefoperazone. Mice treated with either 5Abx plus clindamycin or cefoperazone alone were susceptible to *C. difficile* colonization and ultimately infection.
Conclusion: Resistance to *C. difficile* colonization directly correlated with levels of SCFAs in the murine cecum. Future experiments include identifying other metabolites that make up the gut metabolome, the collection of all metabolites in the murine intestine, when mice are resistant to *C. difficile* colonization and after antibiotics administration. These experiments will help define functional changes to the gut microbiota induced by antibiotics that mediate susceptibility to CDI.

CLOSTRIDUM DIFFICILE CLINICAL ISOLATES EXHIBIT STRAIN-SPECIFIC MOTILITY AND AGGLUTINATION, AND DIFFERENTIAL EXPRESSION OF FLAGELLAR PROTEINS

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Introduction and Rationale: *Clostridium difficile* (CD) causes antibiotic-associated diarrhea (CDI), and epidemic-associated *C. difficile* strains (EA-CD) have spread worldwide in the past 10 years. These strains are linked with severe disease manifestations and high rates of relapsing infection; however, the biological basis for this increased virulence remains largely unknown. No obvious genetic acquisitions/deletions have been identified in EA-CD strain genomes. Therefore, we hypothesized that differential gene expression was responsible for the increased virulence.
Methods and Results: We used comparative proteomics (iTRAQ) to identify differentially abundant proteins in 13 EA-CD strains, compared to a genetically-related, non-EA-CD "ancestral" strain. Multiple proteins with altered abundance were identified in the EA-CD strains; these included flagellin (FliC), the flagellar cap protein (FliD), and flagellar motor/rod molecules (FliM, FlgG). CD flagella (15-24nm in diameter) were visualized in peritrichous orientation by high-resolution electron microscopy, and phenotypic studies revealed that CD strains exhibited profound variations in motility and agglutination. DNA sequence analyses revealed differences in gene content of EA-CD flagellar regions, correlatable with alterations in visualized flagellar architecture. Multiple flagellar genes were disrupted (body, cap and motor; 15 mutants total) resulting in non-motile phenotypes, and increased sedimentation rates, irrespective of strain background. Mutants are currently being assessed for virulence in the hamster model of acute CDI, and a mouse model of CD colonization.
Conclusion: Flagellar-mediated motility varies between EA-CD strains, and is mediated by FliC. This molecule likely critical to CD colonization via its ability to alter sedimentation and thus host-cell signaling proficiency to CD, and its contribution to the increased virulence of EA-CD strains warrants further investigation.

PII-29

EPIDEMIC-ASSOCIATED *CLOSTRIDIUM DIFFICILE* STRAINS EXHIBIT INCREASED RESISTANCE TO MAMMALIAN CATIONIC ANTIMICROBIAL PEPTIDES

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Background and Rationale: *Clostridium difficile* (CD) is a leading cause of hospital-acquired diarrhea. Recent outbreaks of *C.difficile* infection (with increased morbidity and mortality) have involved epidemic-associated (EA) strains. Cationic antimicrobial peptides (CAMPs; e.g. human LL-37 & sheep SMAP-29) contribute to gut innate immunity by interacting with, and disrupting the negatively-charged bacterial cell-membrane. Some pathogens have evolved specific defense mechanisms to evade CAMP-mediated killing. We hypothesized that increased resistance to CAMPs contributes to the more persistent infections associated with EA-CD strains.
Methods and Results: Minimum inhibitory concentrations (MIC) of LL-37 and SMAP-29 were determined for a panel of EA and non-EA CD strains using a standard broth microdilution assay. CAMP activity on CD was visualized using electron microscopy (EM) of LL37-exposed bacteria. CAMP killing-kinetics was evaluated by determining surviving CD throughout a 24h exposure to LL-37. Inducible CAMP resistance was evaluated by testing sensitivity of CD strains to LL-37 with and without pre-exposure to sub-inhibitory concentrations of peptide. Quantitative proteome changes in LL37-exposed CD were determined using mass spectrometry. Compared to non-EA strains, EA-CD isolates displayed increased resistance to LL-37 and SMAP-29. EM of LL-37-exposed CD revealed surface distortion characteristic of CAMP-mediated killing. LL-37 inhibited CD growth in a dose-dependent manner. Exposure of CD to sub-inhibitory LL-37 resulted in increased resistance to the CAMP. Mass-spectrometry analyses revealed proteome alterations, including the increased expression of peptidoglycan-altering proteins in CD exposed to sub-inhibitory LL-37.
Conclusion: The increased CAMP resistance of EA-CD likely contributes to gut persistence via subversion of early, and innate immune responses.

ADHERENCE OF *CLOSTRIDIUM DIFFICILE* TO EPITHELIAL CELLS

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C. difficile is an important pathogen of the gastrointestinal tract and common with most pathogenic bacteria, adherence by *C. difficile* is a prerequisite to the infection and pathology. Adherence of *C. difficile* has been shown to vary with the amount of toxin produced by different strains, with highly toxigenic strains being most successful at mucosal colonisation, and also with the state of differentiation of cell lines, with lack of adherence to undifferentiated colorectal cells. Several adhesins have been identified in *C. difficile* including flagella and surface-layer proteins. The primary aim of this study was to investigate the adherence of five *C. difficile* strains of varying toxigenicity—the hypervirulent ribotype 027, ribotypes 001 and 106, and reference strains VPI 10463 and 630 (ribotype 012)—to different epithelial cell lines—Vero cells, Caco-2 cells and non-mucus secreting and mucus-secreting HT29 cells. Adherence assays were performed with standardised exponential phase cultures of the five *C. difficile* strains and the differentiated epithelial cells for 3 h at 37°C under anaerobic conditions. The secondary aim was to study the extent to which surface-layer proteins and flagella contribute to this attachment to epithelial cells. Adherence-inhibition assays were performed by exposing the cell lines to standardised amounts of surface-layer proteins and flagella before incubation with the cultures. The five *C. difficile* strains adhered to all the cell lines; the extent of binding was independent of cell type but dependent on the strain of *C. difficile*, and the presence of mucus did not enhance adherence. VPI 10463 showed maximum adherence to all the cell lines, while strain 630 was the least adherent strain. Of the epidemic isolates, ribotype 027 exhibited the greatest ability to bind to epithelial cells, followed by ribotypes 106 and 001, respectively, showing that current epidemic strains have an advantage over the historic strain 630. Also, among these strains, adherence correlated with toxin production in the exponential phase. By inhibition, assays flagella and surface-layer proteins of the five strains were found to contribute almost equally to the adherence of the strains from which they were extracted, confirming their role in adherence.

PII-31

CYTOKINE PRODUCTION BY A MACROPHAGE CELL LINE IN RESPONSE TO PROTEINS OF *CLOSTRIDIUM DIFFICILE*

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C. difficile is a major cause of nosocomial diarrhoea. The toxins produced by *C. difficile* are responsible for the characteristic pathology observed in CDI, but several surface-associated proteins of *C. difficile* are also recognised by the immune system and could modulate the immune response in infection. The aim of this study was to assess the induction of cytokines in a macrophage cell line in response to different antigens prepared from five *C. difficile* strains: the hypervirulent ribotype 027, ribotypes 001 and 106 and reference strains VPI 10463 and 630 (ribotype 012). The antigens used were the surface-layer proteins, flagella, crude preparations of heat-shock proteins induced at 42°C and 60°C and culture supernatants of the five strains collected in the exponential and stationary phases of growth. THP-1 cells differentiated with PMA were challenged with the surface-associated proteins for 24 h and shocked with the culture supernatants for 3 h at 37°C under aerobic conditions. Supernatants were collected at different time-points to measure the production of six cytokines: TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-12p70 by in-house ELISAs developed for the same. The production of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-8, and IL-12p70 was observed in response to the surface-associated proteins; IL-10 was not detected. High levels of TNF-α, IL-1β, and IL-8 were detected in response to challenge with culture supernatants. The immune response triggered by the surface-associated proteins was independent of the strain from which the antigens were derived, suggesting that these proteins do not contribute to the increased virulence observed in the hypervirulent ribotype 027 or ribotypes 001 and 106. There was no inter-strain difference observed in response to the culture supernatants of the tested *C. difficile* strains but this was perhaps due to toxicity induced in the macrophages by large amounts of toxin A and toxin B.

ANTIBIOTIC RESISTANCE PATTERNS IN *CLOSTRIDIUM DIFFICILE* 027, 053, AND OTHER TOXIGENIC AND NONTOXIGENIC RIBOTYPES IN SOUTHWEST VIRGINIA

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Monitoring the minimum inhibitory concentration (MIC) of therapeutic and inciting antibiotics will provide a baseline for the early detection of changes with the potential to impact the epidemiology of *C. difficile*. 558 consecutive fecal samples from in, out, and nursing homes patients, were submitted to a southwestern Virginia hospital laboratory. Anonymous, unlinked, excess material from each was screened at TechLab for the presence of *C. difficile*. 131 isolates were recovered and ribotyped. The MIC of metronidazole, vancomycin, rifampicin, moxifloxacin, clindamycin, and erthyromycin were measured using the Etest. All ribotype 027 and 053 isolates were tested for *ermA* and *ermB* by PCR. 131 isolates generated 29 ribotypes. 37% of all isolates were 027. 053 and 014 were 11% and 10%, respectively. 26% of isolates, an unexpectedly high frequency, were from six non-toxigenic types. No isolate of any ribotype was resistant to metronidazole. None was resistant to vancomycin, although 027 isolates had a slightly higher MIC_{50} and MIC_{90} (3 and 4 µg/mL respectively) than other ribotypes (1 and 2 µg/mL). 027 and 053 isolates had an elevated rifampicin MIC_{90} (both >32 µg/mL), although not all isolates were resistant. For all other isolates and ribotypes, the MIC_{90} was 0.003 µg/mL. All 053, and all but one 027, isolates were resistant to moxifloxacin. All 053 isolates were erythromycin and clindamycin resistant, whereas only 33% of 027 isolates were resistant. Based on MIC's of moxifloxacin, rifampicin, clindamycin, and erthyromycin, coupled with the presence or absence of *ermA* and *ermB*, we identified two main phenotypes among 027 isolates and one among 053. Single isolates of either ribotype may represent other phenotypes. The prevalence of 027 and 053 in southwest Virginia is 37% and 11%. The data forms part of the baseline to our continuing surveillance of *C. difficile* in southwest Virginia.

Anaerobe 2012

845 *POSTER SESSION II:* CLINICAL ASPECTS OF ANAEROBIC INFECTIONS

Posters will be presented in Poster Session II
Saturday, June 30 845-945.

SULFATE-DEPENDENT ANAEROBIC HYDROCARBON DEGRADATION IN ESTUARINE SEDIMENTS (RIVER TYNE, UK)

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Anaerobic biodegradation of petroleum hydrocarbons can be catalyzed by sulfate-reducing bacteria (SRB). In order to understand this process, River Tyne sediment microcosms were amended with North Sea crude oil. Pore water sulfate decreased from 9 to 1 mM in the top 12 cm of triplicate sediment cores, therefore sediment from 4-12 cm was pooled and inoculated in 171 experimental microcosms. Brackish medium (60 ml) was amended with crude oils and nutrients under eight conditions: 1-3, crude oil with ammonia and phosphate added at low (4.7 and 1.5 mM), medium (56 and 5.6 mM) and high (100 and 10 mM) concentrations; 4-6, corresponding no-oil controls; 7, "topped" crude oil from which low molecular weight (LMW) volatile hydrocarbons had evaporated; 8, autoclaved control with crude oil. Sulfate depletion was faster with "topped" oil, compared to crude oil containing volatile hydrocarbons after 231 days of incubation indicating that volatile components of the crude oil may partially inhibit sulfate-reduction. nC_{17}:pristane ratios demonstrated small but significant levels of crude oil biodegradation after 231 days of incubation only when high levels of inorganic nutrients were present. Sulfate-reduction was much slower on the no-oil controls. Enrichment of organisms carrying alkylsuccinate synthase genes was evident after 105 days of incubation with crude oil whilst enrichment of organisms carrying benzylsuccinate synthase genes was evident after 161 days of incubation with crude oil, suggesting that alkanes are degraded prior to aromatic compounds. The results suggest that crude oil degradation was driven by sulfate-reducing communities.

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ANAEROBIC BACTERIA IN PERFORATED CORNEAL ULCERS: RESULTS OF 17 CASES

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Bacterial corneal ulcers generally follow a traumatic break in the corneal epithelium, thereby providing an entry for bacteria. The traumatic episode may be minor, such as a minute abrasion from a small foreign body, or may result from causes as tear insufficiency, malnutrition, or contact lens use. In this study we report the anaerobic bacteria that we isolated from corneal scrapings of 11 cases with ulcerative keratitis and 6 cases with endophthalmitis.

Corneal scrapings were taken by the pinpoint of 3 syringes, one of the pinpoint containing a specimen was layed on a slide. The specimen of the second pinpoint was collected on 1 ml of PBS for PCR analyses and was kept at -70 C° until the test day. The third pinpoint was plunged on 3 ml of sodium thyoglycolate broth enriched with hemin and sheep blood and was incubated 72 hours on anaerobic conditions obtained with Anaero-Gen (Oxoid & Mitsubishi Gas Company) in anaerobic jars (oxoid). In the 24th hour of this incubation, one or two loops of sodium thyoglycolate broth was subcultured on blood agar which was incubated on aerobic conditions for the isolation of aerobic bacteria, then the anaerobic incubation of the broth was prolonged until 48 hours. For the isolation of anaerobic bacteria, one or two drops of the enriched broth was inoculated on phenylethyl alcohol anaerobic agar and on kanamycin vancomycin anaerobic agar. These media were incubated 5 days on anaerobic conditions. Preliminary identification of anaerobic bacteria was made and typical colonies were taken on 1 ml of PBS for PCR analyses. Multiplex PCR (FC Biotech Anaerobic Multiplex PCR kit) was used both for the identification of anaerobic bacteria existing in scrapings specimens and for the identification of typical colonies .

During two years, corneal scrapings of 182 cases were studied. Anaerobic bacteria were isolated from 11 (6%) cases with ulcerative keratitis and from 6 (3.2%) cases with endophthalmitis. Multiplex PCR identified *P. acnes* in 5 cases with ulcerative keratitis and in 3 cases with endophthalmitis. *Peptostreptococcus* sp. was identified in 5 cases with ulcerative keratitis, 2 of them with *P. acnes* and also it was identified in 2 cases with endophthalmitis. Multiplex PCR identified *Prevotella* sp. in 3 cases with ulcerative keratitis and in 2 cases with endophthalmitis, it was with *Peptostreptococcus* sp. in one of the cases with endophthalmitis. This PCR identification of typical colonies matched with the results of the identification from scrapings.

We concluded that to determine the presence of anaerobic bacteria in corneal scarapings of cases with ulcerative keratitis and endophthalmitis have a great importance for the order of an appropriate treatment. Gram stain smears results could be effective for the preliminary decision of the antibiotherapy, but the isolation and identification of anaerobic bacteria and the run of the infection under the appropriate antibiotherapy will impress the decision of the ophthalmologist about surgery or to continue treatment.

SMOOTH CONTACT LENS USAGE INFLUENCES THE POPULATION DENSITY OF *PROPIONIBACTERIUM ACNES* IN CONJUNCTIVAL FLORA

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Propionibacterium acnes is one of the predominant organisms residing on the ocular surface. The purpose of this study is to determine the alternation in population density of *P. acnes* after usage of frequent replacement daily wear hydrogel and silicone hydrogel contact lenses.

Swabs was taken from 60 patient's conjunctiva before and after usage the first time hydrogel contact lenses and from 40 patient's conjunctiva before and after usage the first time silicone hydrogel contact lenses. Swabs were inoculated on phenylethyl alcohol anaerobic agar, prepared with Scaedler agar as a basal medium and enriched with 5% sheep blood and were incubated 72 hours on anaerobic conditions obtained with Anaero-Gen (Oxoid & Mitsubishi Gas Company) in anaerobic jars (oxoid). *P. acnes* was determined upon their colony morpholgy, their characteristics view on Gram stained smears and also by Multiplex PCR (FC Biotec Anaerobic Multiplex PCR kit). The population density of *P. acnes* was determined semiquantitatively by the number of *P. acnes* colony in each quadran of the petri dishes containing phenylethyl alcohol anaerobic agar.McNemar test was used for statistical analyses

P. acnes was determined by PCR in 34 of 60 patient's conjunctiva before usage of hydrogel contact lenses with a population density of (1+) in 17.6% ,(2+) in 70.6%, (3+) in 11.8% and (4+) in 0%. Additionnaly, *P. acnes* was determined by PCR in 24 of 40 patient's conjunctiva before usage of silicone hydrogel contact lenses with a population density of (1+) in 33.3%, (2+) in 58.3%, (3+) in 8.4 % and (4+) in 0%. After one month usage of contact lenses, patients were called to the contact lens department of the ophthalmology clinic and conjunctival swabs were taken. *P. acnes* was determined by PCR in 46 of 60 patient's conjunctiva after usage of hydrogel contact lenses with a population density of (1+) in 0% (2+) in 13.1%, (3+) in 30.4 %, and (4+) in 56.5%. Additionnaly *P. acnes* was determined by PCR in 34 of 40 patient's conjunctiva after usage of silicone hydrogel contact lenses with a population density of (1+) in 0%, (2+) in 11.8%, (3+) in 23.5%, and (4+) in 64.7%. Comparing with the population densities determined before usage, a statistically significant differance was found in conjunctival population density of *P. acnes* which was determined as (3+) and (4+) after usage of hydrogel contact lenses ($p<0.05$) and which *was* determined as (4+) after usage of silicone hydrogel contact lenses ($p<0.05$).

In conlusion, our results indicated that the conjunctival population density of *P. acnes* increases after usage of frequent replacement daily wear hydrogel and silicone hydrogel contact lenses. The potential of biofilm formation of *P. acnes*, the chemical and physical characteristics of lenses and the user's poor knowledge about lens usage might influence this result.

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PII-36

ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL PATHOGENS IN OTITIS MEDIA

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The antibiotic susceptibility of aerobic and anaerobic bacteria isolated from patients with otitis media was done by the disk diffusion and E-test methods. The choice of antibiotics was based on availability in our environment, clinical outcome, and rare prescriptions following relapses with first-line drugs. Amongst the aerobes, streptococci, and haemophili were highly susceptible to all the antibiotics including amoxicillin. Strains of *Staphylococcus aureus*, *Escherichia coli,* and *Moraxella catarrhalis* resistant to amoxicillin and the cephalosporins were sensitive to amoxicillin-clavulanic acid combination, ciprofloxacillin, and tigecycline. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* showed high level resistance to the β-lactam and β-lactam- β-lactamase stable drugs; however 85-98% were sensitive to either ciprofloxacillin or tigecycline. The anaerobes were generally sensitive to amoxicillin-clavulanic acid combination and metronidazole. Resistance occurred in 10% of *Bacteroides thetaiotaomicron*, *Bacteroides ureolyticus*, *Prevotella melaninogenica,* and *Peptostrepococcus magna*. Amoxicillin activity against the anaerobes was low (< 10% in most cases), except for anaerobic cocci with activity as high as 80%. Activity of the cephalosporins for the anaerobes was staggering with cefuroxime having the highest activity (70-100%). The high level of resistance against the β-lactam antibiotics was associated with β-lactamase production by the pathogens.

OCCURRENCE OF OTITIS MEDIA IN CHILDREN AND ASSESSMENT OF TREATMENT OPTIONS

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Otitis media is a more frequent occurrence in children, and the disease may progress from acute to chronic state, if appropriate, intervention is not initiated. 212 children 6 months to 10 years were examined and treated for otitis media in a 13 month hospital based study. Exclusion criterion was history of antibiotic therapy 2 months to presentation at clinic. Acute otitis media (AOM) was diagnosed in 130 of the patients (25 in 6 months and 46 in 7 months to 12 months infants; 28 in children older than 1 year to 2 years; 19 in age group >2 years to 5 years; and 12 for children between 5 to 10 years) and 82 cases were of chronic otitis media (7 in 6 months and 30 in 7 months to 12 months infants; 15 in children older than 1 year to 2 years; 12 in age group >2 years to 5 years; and 18 for children between 5 to 10 years). Patients with AOM were given paracetamol only during the period of pain and effusion when present. Relapses occurred in 25 to 41.7% of cases with highest incidence in the age groups 7 months to 1 year and 2 to 5 years. Cases of recurrence were investigated bacteriologically and treated with amoxicillin or augmentin (ampicloxacillin + clavulanate). Chronic otitis media patients were assigned into three treatment groups; amoxicillin and augmentin prophylactic groups: (27 patients each) and a treatment group based on culture and antibiotic susceptibility (CAS) test results (28 patients). Recovery occurred in 19, 24 and 27 patients in the amoxicillin, augmentin, and CAS groups respectively with relapses seen only in the amoxicillin (5 cases) and augmentin, (2 cases) groups. Infection persisted in 8, 3, and 1 patient in the amoxicillin, augmentin and CAS treated patients. The high rate of recurrence in AOM and the success rate with antibiotic therapy for both AOM and COM suggest that antibiotics should be considered especially when culture and sensitivity patterns are established.

PII-38

CLOSTRIDIUM DIFFICILE INFECTION (CDI) IN SPINAL CORD INJURY/DISORDER (SCI/D) PATIENTS: TRENDS OVER TIME AND RISK FACTORS

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Purpose: Little is known about CDI in patients with SCI/D. Our objective was to characterize the burden of healthcare facility-onset (HCFO), HCF-associated (HCFA) CDI in Veterans with SCI/D and examine risk factors associated with CDI.
Methods: We conducted a retrospective, longitudinal analysis of 9 years (2002-2010) of available medical, pharmacy, and laboratory data in Veterans with SCI/D. HCFO/HCFA CDI was defined as a positive test >48 hours after admission and during hospitalization. Recurrence was defined as CDI that occurred >2 and ≤8 weeks after the onset of the previous episode. Severity was defined by SHEA/IDSA guidelines. Risk factors were assessed using random effect Poisson regression, where relative risks (RR) and 95% Confidence intervals (CI) were calculated.
Results: About 15,000 Veterans with SCI/D were hospitalized in VA during the study time period. Of the 1,946 cases of HCFO/HCFA CDI that occurred, 22.9% were recurrences, and 31.2% were severe complicated; the overall incidence rate of new cases was 6.9/10,000 bed days of care (BDOC). The rate of CDI significantly increased between 2002 and 2005, and declined to 3.2/10,000 BDOC by 2010. Differences in rates were seen in age, region of the country, and SCI/D characteristics. Antibiotic use was associated with developing CDI (RR=11.1, 95% CI 9.1-13.5), exposure to PPIs and/or H2 blockers were associated with a RR=2.0 (95% CI 1.8-2.3), and 30-day history of an invasive respiratory procedure was associated with nearly 4x the risk of developing CDI.
Conclusions: HCFO/HCFA CDI is prevalent and associated with typical risk factors in patients with SCI/D. A significant portion of cases had severe complicated illness (mostly due to hypotension); this may reflect either true illness severity and/or differences in blood pressure in SCI/D patients. Guidelines for severity may need to differ in this population. These data can be used to plan prevention strategies and to improve ongoing initiatives to prevent CDI.

RISK FACTORS FOR THE DEVELOPMENT OF *C. DIFFICILE* INFECTION (CDI) IN CANCER PATIENTS, CANCER INSTITUTE AND HOSPITAL, CHINESE ACADEMY OF MEDICAL SCIENCES, BEIJING, CHINA BETWEEN APRIL AND DECEMBER 2011

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Antibiotic therapy is considered the most important drug leading to the development of CDI. Cancer chemotherapy is an underappreciated risk factor in the development of CDI. The objectives were 1) to determine the rate of CDI in oncology patients with hospital acquired diarrhea (HAD) in a Chinese cancer hospital; 2) to investigate the relative importance of cancer chemotherapy versus antibiotics in the development of CDI in cancer patients.

Study population consisted of patients ≥ 18 years of age in the Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing, China between April and December 2011. CDI was defined in a case of HAD when *C. difficile* toxin A and/or toxin B was detected by EIA in stools. A total of 277 patients with HAD were enrolled. Forty-one consecutive stools from patients with CDI and the next patient enrolled with a negative *C. difficile* toxin test was enrolled in comparison study.

Two hundred and seventy-seven patients with HAD were studied and 49 (18%) were diagnosed as having CDI.

Variable		*C. difficile* toxin A/B test		P-value
		Positive (N=41)	Negative (N=41)	
Age (years)	Mean±SD	56±16	56±14	0.6457
	Range	23-78	27-84	
Gender (Male)		23 (56%)	28 (68%)	0.2548
Antibiotic given		1 (2%)	1 (2%)	1.0000
Colon Cancer Diagnosis		16 (39%)	11 (27%)	0.0711
Other Non-GI Related Cancer		16 (39%)	9 (22%)	0.0631
GI-Related Cancer but not Colon Cancer		4 (10%)	11 (27%)	0.0456
Lymphoma		2 (5%)	4 (10%)	0.3664
Cancer chemotherapy given		16 (39%)	9 (22%)	0.0290
Length of chemotherapy before diarrhea (days)	Median	39	22	0.0391
	Range	1-276	1-128	

It was demonstrated in the present study that anti-tumor chemotherapeutic agents were a more important risk factor for CDI than antibiotics in a cancer population. Receipt of chemotherapy and the length of the treatment were major determinates of CDI. Patients with colon cancer experienced high rates of HAD and CDI. Cancer patients, especially colon cancer patients, developing HAD should be screened for CDI.

PII-40

USE OF NON-PATHOGENIC ENGINEERED CLOSTRIDIA SPORES AS A DELIVERY VECTOR FOR TOXIC GENE PRODUCTS TO THE TUMOUR

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The aim of Clostridial-directed enzyme pro-drug therapy (CDEPT) is to use modified, non-pathogenic bacteria able to produce pro-drug converting enzymes inside the tumour to convert non-harming pro-drugs into toxic derivatives eradicating tumour cells.

Successful and widely available cancer treatment still remains a challenging problem worldwide. Many efforts have been put into researching alternative methods of administering effective therapy that can terminate the development of cancer cells. One of these is the use of non-pathogenic strains of the bacterial genus Clostridia as a delivery vector of currently available anti-cancer drugs directly to tumour cells.

It has previously been shown that these obligate anaerobes can successfully colonize hypoxic regions which are present in solid tumours and not in normal tissues. Clostridial spores injected into a tumour-bearing organism will only germinate within those low-oxygenated regions. The combination of this knowledge with recent studies of pro-drug converting enzymes created the new alternative cancer treatment pathway CDEPT.

We have used state of the art technology to introduce a synthetic gene encoding a novel nitroreductase enzyme into the genome of *Clostridium sporogenes* and confirmed its activity. Additionally, we successfully improved enzyme expression on the molecular level. Furthermore, we have sequenced the genome of *C. sporogenes* NCIMB 10696 using Roche 454 Platform and prepared a first partially annotated draft.

To date, the constructed pro-drug converting enzyme-bearing *C. sporogenes* strain has been characterized and its enzymatic activity as an equivalent of effective pro-drug conversion has been tested. An *in vivo* tumour colonization study was performed showing major therapeutic effects in the mouse model.

MICROBIOME TRANSPLANTATION APPARENTLY REVERSES SYMPTOMS OF LATE ONSET AUTISM: A CASE STUDY

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Objective: To determine if Fecal Microbiome Transplantation (FMT) can reverse clinical features of late onset autism.
The case: A 5 ½ year old female, Caucasian, with normal development and reading, writing, and academic skills advanced for her age developed a febrile illness with skin rash and headache in October 2010. After 2 weeks, the illness gradually cleared but onset of Obsessive Compulsive Disorder, ADHD behavior, separation anxiety, irrational fear, night terrors, choreiform movements, joint pains, deterioration of language, and memory skills to a 2-3 year old level followed and persisted. A diagnosis of PANDAS (pediatric autoimmune neuropsychiatric disorder associated with group A streptococcus) was made on clinical grounds without supporting microbiologic evidence. In November and December 2010, treatments included Beta Lactam antibiotics intravenously, orally and intramuscularly (Bicillin), corticosteroids and intravenous gamma globulin without demonstrable benefit. Choreiform movements diminished. A 10 day course of amoxicillin/clavulanate appeared to improve the severity of symptoms. Oral vancomycin 125 mg QID was commenced in January 1, 2011 with clinical improvement of symptoms and IVIG 40 gm was given every 6 weeks on 3 occasions (mid-February, end of March, and early May 2011). It was decided to discontinue Vancomycin after 3 months (March 31), but symptoms reappeared within 6 days, resulting in resumption of vancomycin and resolution of symptoms within a 5-7 days. A second attempt at discontinuing vancomycin in mid-June also resulted in recurrence of autistic symptoms. At this point FMT was sought on the basis that vancomycin might be suppressing a pathogen(s) and that the damaged microbiome associated with vancomycin treatment was not able to prevent regrowth of the pathogen.
Investigation and treatment plan: A comparison of the microbiology of the feces on vancomycin (until August 1, 2011), after recrudescence of autistic symptoms for the 3rd time (Aug. 5), retreatment with vancomycin x 7 days followed by fecal microbiome transplantation August 12, 2011. The procedure involved a cleansing enema, followed by 400 ml of a fecal slurry per rectum with retention balloon, followed by an equivalent of 100 gm of fecal microbes recovered by serial centrifugation, condensed into 12 triple encapsulated gelatin capsules p.o. 18 hours after the fecal PR slurry.
Result: The autistic symptoms did not recur for the next 4 months. In late December 2011, follow antibiotic exposure, mild symptoms recurred, which was arrested by a repeat oral fecal microbiome administration. Quantitative differential and selective aerobic and anaerobic cultures of the samples recovered 280 colony types different between the first and second samples. Clostridial organisms appeared to predominate; sequencing of unknown types showed that *Clostridium bolteae* 10^7/gm wet weight predominated in the second sample. The samples were sent to E. Allen-Vercoe for high through-put sequencing (results pending).
Conclusion. In this case study, vancomycin treatment appeared to reverse autistic behavior, and FMT appear to prevent recrudescence of symptoms associated with discontinuing vancomycin. It is unclear if *Cl. bolteae* is a causal agent. Further studies are required.

PII-42

STUDIES ON MOLECULAR INTERACTIONS OF *FINEGOLDIA MAGNA*

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In the present study, molecular mechanisms between *Finegoldia magna* and the host of importance for infection will be characterized.

The anaerobic Gram-positive bacterium *F. magna* is part of the normal flora on skin and mucous membranes. This bacterium can also cause infections, such as soft tissue abscesses, wound infections and bone/joint infections, and is often found in chronic wounds, such as leg ulcers. Two surface molecules, the enzyme SufA and the adhesion molecule FAF, are expressed by most *F. magna* isolates.

Overlay experiments with human epidermal skin extracts and FAF followed by mass spectrometry analysis identified Galectin-7 and Histones 2B and 4 as ligands for FAF. Galectin-7 is a lectin associated with differentiation and development of pluristratified epithelia and plays a crucial role in re-epithelialization of epidermal wounds. FAF binding to Galectin-7 might interfere with this process and delay wound healing. Purified Galectin-7 was found to bind to the FAF-expressing strain ALB8 and further binding studies using radiolabelled Galectin-7 showed that several other FAF-expressing *F. magna* isolates bind the lectin. The role of *F. magna* interaction with Galectin-7 during infection will be further investigated by carrying out wound healing assays on keratinocytes. Histones package and order DNA into structural units (nucleosomes) without compromising the crucial properties of DNA. They are also known to have an antibacterial function. Purified Histones 2B and 4 were found to bind to purified FAF. In addition, both histones had an antimicrobial activity on an *F. magna* strain that does not express FAF. Addition of exogeneous FAF blocked the antibacterial activity.

These results reveal more of the mechanisms that *F. magna* uses during infection. By binding to histones through FAF, it is protected from their antimicrobial activity and by interacting with Galectin-7, it is hypothesised that this delays wound healing and keratinocyte migration. The studies described here will make an important contribution to the knowledge of *F. magna* virulence factors and may contribute to alternative therapeutic remedies for wound infections.

INVESTIGATION OF THE MAIN ANTIBIOTIC RESISTANCES AND THEIR CORRELATION WITH THE PRESENCE OF ANTIBIOTIC RESISTANCE GENES IN CLINICAL *BACTEROIDES* STRAINS

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Objectives: We aimed to study the main anti-*Bacteroides* drug susceptibilities of clinical *Bacteroides* strains and the presence of an extended set of antibiotic resistance genes in order to reveal the situation concerning the rates of molecular antibiotic resistance mechanisms among this group of species.
Methods: Antibiotic susceptibilities of 174 *Bacteroides* (including 132 *B. fragilis*) strains were recorded by agar dilution for ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefoxitin, imipenem, metronidazole, clindamycin, moxifloxacin and tigecycline, and the presence of the *cepA*, *cfxA*, *cfiA*, *ermF*, *tetQ*, *tetX* and *tetM* genes by Real-Time PCR. For ermF, the upstream presence of the IS*4351* element was determined using PCR-mapping.
Results: The resistance prevalences were 98.2, 10.4, 10.3, 17.2, 1.2, 0.5, 32.4, 13.6, and 1.7% for ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefoxitin, imipenem, metronidazole, clindamycin, moxifloxacin and tigecycline, respectively. The most frequent resistance genes were the *cepA* and *tetQ* in 71.3 and 80.5%, respectively, responsible for the most prevalent normal beta-lactam and tetracycline resistances. For *cfxA*, *ermF*, *tetM*, and *tetX* the following prevalences were found: 17.8, 23.0, 0.6, and 10.3%. The IS*4351* element was found in 16 strains (9.2%), and 11 strains co-harboured *ermF* and IS*4351*, but IS*4351* could be mapped to *ermF* genes only in two cases. The frequency of the *cfiA* gene was 11.4 % among *B. fragilis* strains, and no coincidence with *cepA* was seen. The *cepA* gene was more frequent in *B. fragilis* strains (79.5 vs. 45.2) and the *cfxA* gene was more frequent in non-fragilis *Bacteroides* (14.4 vs. 28.6 %). All the tigecycline-resistant strains (n=3) were positive for *tetQ* but negative for *tetX* and *tetM*.
Conclusions: The prevalence of the resistance genes correlated with the corresponding antibiotic resistance rates, except for *cfiA* and the carbapenem resistance. However, further search for other resistance genes and activation mechanisms are needed to account firmly with all the resistant cases.

PII-44

ENVIRONMENTAL CONTAMINATION OF *CLOSTRIDIUM DIFFICILE* IN A RADIOLOGY ULTRASOUND DEPARTMENT

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Aims: To assess the presence of *Clostridium difficile* and the potential risk of transmission to patients and health-care professionals in a high-risk clinical area within a radiology department.
Methods: Contact plates (cycloserine, cefoxitin, egg-yolk: CCEY) were used to sample 35 environmental sites for *C. difficile*. These were designated as high or moderate risk within two ultrasound (US) rooms with the greatest in-patient movement. Blood agar contact plates were also used to calculate overall colony counts in order to assess areas of greatest contamination. Each site was sampled at the start and end of a standard 8 hour working day. Contact plates were then incubated and evaluated according to standard or manufacturer protocols. The blood agar contact plates were divided for both aerobic and anaerobic incubation. This gave a total of 70 CCEY and 140 blood agar plates.
Results: Two of 70 CCEY contact plates were culture positive for *Clostridium difficile*. These were isolated from the curtain rail and under-surface of the patient couch frame belonging to the same cubicle. Both samples were positive for toxins A/B, via enzyme immunoassay, and sensitive to both metronidazole and vancomycin via E-test. The highest overall combined aerobe and anaerobe colony counts were obtained from the patient couches, computer keyboards, floor, US probe holder, US control panel and US gel warmer with a median colony count of 198 (range 123 to 954). The areas demonstrating the greatest increase in colony counts over the course of the day were the US control panel, a US probe, the US gel warmer and the floor.
Conclusions: The study identifies areas, which may be overlooked by current cleaning protocols where *C. difficile* spores can exist and act as a potential source of hospital acquired infections. In addition, potential areas of increased contamination have been highlighted, allowing greater awareness of areas to target during standard decontamination procedures.

RISK FACTORS AND PREDICTORS FOR 30 DAY ALL-CAUSE MORTALITY IN *EGGERTHELLA LENTA* BACTEREMIA

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Statement of Purpose: The risk factors for mortality with *Eggerthella lenta (E. lenta)* bacteremia and sepsis are unclear; this study was done to assess those risks.
Methods: A retrospective chart review was performed on all cases of *E. lenta* bacteremia from 1999-2010. The patients' medical records were reviewed for demographics, underlying co-morbid conditions and laboratory findings.
Results: There were a total of 25 cases of *E. lenta* bacteremia during the study period. All patients had evidence of fever, hypotension, or leukocytosis at the time of the bacteremia. The mean age was 67.1±14.9 (s.d.) years and the most common underlying co-morbid conditions were decubitus ulcers (52%), diabetes mellitus (36%), and solid tumor malignancies (28%). The source of infection was an abdominal source in 44% (11) and a complicated soft tissue infection in 40% (10) of cases. Overall, 36% (9/25) of the patients died within 30 days of the diagnosis, with six dying on the same admission. From univariate analysis, monomicrobial bacteremia, absence of fever on the day of bacteremia, duration of fevers, systolic hypotension, hypoalbuminemia, recent ICU stay and an elevated modified Charlson score were all associated with increased risk of 30 day mortality. From logistic regression, absence of fever on the day of bacteremia (OR=0.66, 95% C.I. 0.44-0.97) and ICU stay (OR=17.37, 95% C.I. 1.31-229.57) were independent predictors of 30 day all-cause mortality.
Conclusions: The most common sources of infection in patients with *E. lenta* bacteremia are abdominal and soft tissue infections. *E. lenta* bacteremia is associated with a high all-cause mortality rate. The absence of fever at presentation and the need for ICU stay were independent risk factors for 30 day mortality.

PII-46

CLOSTRIDIUM DIFFICILE COLONIZATION IN PATIENTS ADMITTED TO TWO DIFFERENT HOSPITALS/WARDS

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*Clostridium difficil*e is recognized as an important nosocomial pathogen and asymptomatic colonized individuals have been suggested as one of possible sources for transmission.

The main objective of our study was to determine carriage rate in all patients admitted to two different wards in two different hospitals in December 2010 and January 2011. Altogether 175 patients were screened, 143 (81, 7%) once and 32 (18, 3%) on multiple occasions (upon admission and subsequently during hospitalization after 1 to 29 days from first sampling). Collected samples were tested for *Clostridium difficile* by in house real-time PCR specific for toxigenic and nontoxigenic *C. difficile* strains and with *C.difficile* specific 16S rDNA. Additionally, an enrichment was performed in selective *Clostridium difficile* broth (Oxoid), supplemented with sodium cholate and lysozyme to increase spore germination, followed by alcohol shock and isolation on selective commercial CLO agar plates (BioMerieux). All isolates were characterized by toxinotyping and PCR ribotyping.

Altogether 22 out of 175 (12, 6%) screened patients tested positive for *Clostridium difficile*. Carriage rate of 9, 9 % was determined in ward with primarily younger patients (1-9 years of age) and 14, 4 % in second ward with primarily older patients (1-90 years of age). Only 16 samples (72, 7%) resulted positive by both, culture and molecular based method. However no major difference was observed in sensitivity of tested methods. Two out of 30 (6, 7%) initially negative patients that were sampled more than once became positive within 5 days. Isolated toxigenic strains (88, 2 % of all isolates) were distributed into toxinotypes 0, IV and XII and multiple PCR ribotypes (5 -9) were identified within a single ward. PCR ribotype 014/020, type frequently isolated from CDI patients and other environments (animals, water, ...) prevailed on one of the wards.

915 *POSTER SESSION III:* DIAGNOSTIC METHODS & MICROBIOLOGY

Posters will be presented in Poster Session III
Sunday, July 1 915-1000.

APPLICATION OF A NOVEL QUANTITATIVE-PCR ASSAY TO INVESTIGATE THE ROLE OF *PROPIONIBACTERIUM* IN THE AETIOLOGY OF PROSTATE CANCER

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Recent studies suggest that *P. acnes* may be a frequent coloniser of prostate tissue, where it is associated with acute and chronic inflammatory changes which could potentially stimulate carcinogenesis. Culture methods for *P. acnes* detection are sub-optimal due to the slow growth of the organism coupled with the lack of sensitivity of end-point PCR, therefore, we have developed a novel quantitative TaqMan® PCR assay to detect and quantify *Propionibacterium acnes* in cancerous prostate tissue.

Our developed assay employs the use of primers that target *Propionibacterium* specific regions of the 16S rRNA gene, in combination with the TaqMan probe which was designed to hybridise with *P. acnes* sequences only. PCR inhibition was eliminated using an established human endogenous retrovirus-3 assay which quantifies human cells present in clinical samples and simultaneously provides normalisation of the *P. acnes* genome count. DNA from a range of bacterial species was used to assess specificity of the assay. The assay was applied to DNA extracted from archived tissue specimens retrieved from prostate cancer patients in the UK. Archived prostate tissue from disease-free patients and non-prostatic tissue controls were compared. Our studies confirm the presence of *P. acnes* DNA in the prostate tissue of UK patients and reveal levels in cancerous prostates significantly higher than those found in control tissue ($p<0.001$).

We have successfully developed an assay to detect *P. acnes* specific DNA in clinical material. The detection limit of the assay is ten genome copies and exhibits no cross reactivity with a panel of other bacterial species. Our results reveal a significant correlation between the infection/colonisation of prostate tissue by *P. acnes* and prostate cancer. *P. acnes* may therefore be a significant driver of inflammation in the prostate and the subsequent carcinogenic changes.

PIII-2

DEVICE FOR HYDROGEN SULFIDE REMOVAL IN ANAEROBIC CHAMBERS

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Purpose: We desired to develop and feasibility test a column for the removal of hydrogen sulfide (H_2S) from enclosed anaerobic chambers. Build-up of H_2S from the culturing of H_2S-producing bacteria causes undesirable deposits, decreases palladium catalyst life, harms expensive electronics, and enables sulfuric acid damage. Such a column is needed as other methods for H_2S removal, such as bubbling through a silver sulfate solution, lack desired capacity, can be difficult to use, are inefficient at H_2S removal, and/or lack any indication when capacity is reached and are no longer functioning.
Summary of Methods and Results: The hydrogen sulfide removal column (HSRC) functions by recirculating the anaerobic chamber atmosphere through layers of defined media at a controlled rate. This facilitates chemical interactions that leave the output free of H_2S, based on an H2S-specific sensitive color-change indicator. Various media were examined including impregnated carbons, activated carbon, triazine-coated zeolite, and potassium permanganate impregnated media. Many other important design considerations were also addressed in the design and application of the HSRC, including temperature, humidity, use in a contained environment, detection of H_2S, function in an anaerobic atmosphere, byproducts of reaction with H_2S, disposal of spent media, H_2S capacity, chamber atmosphere circulation, and overall HSRC flow characteristics to optimize performance. The HSRC was run continuously without any maintenance under constant exposure to H_2S for a 6-month period and had remaining capacity based on H_2S indicators at the outlet. Inside the chamber, H_2S levels reached as high as ~10ppm during growth of *C. difficile* without the HSRC. Within 10 minutes, the HSRC could lower this to a level undetectable with sensitive lead acetate indicators.
Conclusions: We have successfully completed the initial design and feasibility testing of a column for H_2S removal in an anaerobic chamber that is efficient and also easy to use with an indicator of ongoing function. The HSRC also shows high capacity for H_2S,based on long-term removal of routine levels of H_2S produced by *C. difficile* cultures.

ISOLATION AND PRESUMPTIVE IDENTIFICATION OF *FUSOBACTERIUM NECROPHORUM* FROM THROAT SWABS

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Studies have shown that *Fusobacterium necrophorum* could be one of the leading causes of acute tonsillitis and peritonsillar abscess. This has led to a renewed interest in performing throat cultures anaerobically for the isolation of *F. necrophorum*, as well as a need for a media designed for isolation and presumptive identification of the organism. A PRAS Egg Yolk Agar was used as the base for our identification media, due to the presence of a lipase reaction with *F. necrophorum*. 1.0mL/L of vancomycin was added as well kanamycin in concentrations of 1.0mL/L, 2.0mL/L, and 4.0mL/L. All 3 formulations were tested with *Fusobacterium necrophorum* (ATCC 25286), *Fusobacterium nucleatum* (ATCC 25586), *Clostridium perfringens* (ATCC 13124), *Bacteroides fragilis* (ATCC 25285), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 7002), and *Staphylococcus aureus* (ATCC 25923). At 1.0mL/L of Kanamycin 3rd and 4th quadrant growth was observed in all organisms with the exception of *S. aureus* which exhibited no growth. At 2.0mL/L inhibition of *F. nucleatum* was observed along with the lack of growth from *S. aureus*. All other organisms exhibited strong growth into the 4th quadrant. At 4.0mL/L all organisms were inhibited with the exception of *F. necrophorum* and *B. fragilis*. Only *F. necrophorum* exhibited a lipase reaction. Based on this study, a PRAS Egg Yolk Agar containing 1.0mL/L vancomycin and 4.0mL/L kanamycin (EYKV) will aid in isolating and providing presumptive identification of *Fusobacterium necrophorum* within 24 to 48 hours of acquiring an acceptable anaerobic specimen.

PIII 4

DEVELOPMENT, OPTIMIZATION, AND QUALIFICATION OF A SENSITIVE HIGH THROUGHPUT CELL-BASED NEUTRALIZING ANTIBODY ASSAY FOR *CLOSTRIDIUM DIFFICILE* TOXIN A AND B

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Toxins A and B are the major virulence factors of disease caused by *C. difficile* and, thus, are attractive targets for vaccine development. A robust High Throughput (HTP) neutralization (neut) assay for toxin A and B is required to measure the functional antibodies in both preclinical and clinical samples.
Methods: Anti-toxin neutralizing activity is measured by inhibition of toxin-mediated cytotoxicity by antibodies in a test sample. Briefly, a 96-well culture plate was seeded with human fibroblast cells (IMR-90). The following day, equal volumes of antiserum and toxin were mixed together. After incubation for 90', the mixture was transferred to the cell culture plate. Incubation at 37°C was continued for 72h after which the monolayer was treated with CellTiter-Glo® (Promega) reagent generating luminescent signal proportional to the amount of cellular ATP. The neutralization antibody concentrations in neut units/mL were determined from an antitoxin A and B Reference Standard.
Results: Traditional methods measure the level of cytotoxicity by observing the extent of cytopathic effect on the cell monolayer by microscopic evaluation and assigning an arbitrary score. This method is subjective, tedious and not amenable to automation. We used CellTiter Glo® to determine the number of viable cells based on the measurement of cellular ATP. A multivariate Design of Experiment (DOE) was used to optimize assay conditions during development. Although toxin A and B share sequence identity, the assay was shown to be specific to the type of toxin used. The assay showed excellent accuracy and precision within the quantitative range of 21–176 and 40–1280 neut units/mL for toxin A and toxin B, respectively.
Conclusions: A specific and reliable HTP neut assay was developed and qualified to measure anti-toxin A and B antibody responses. The assays were shown to be robust and to reproducibly measure functional anti-toxin antibodies levels in serum samples from preclinical and clinical studies.

DEVELOPMENT AND EVALUATION OF A DOUBLE MULTIPLEX REAL-TIME PCR METHOD FOR DETECTION OF THE *CLOSTRIDIUM DIFFICILE* TOXIN A, TOXIN B AND BINARY TOXIN A

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C. difficile is the most common cause of infectious antibiotic-associated diarrhea with symptoms ranging from mild diarrhea to severe pseudomembranous colitis in hospitalized patients. Rapid identification of *C. difficile* is important for patient management and prompt epidemiological interventions. The aim of the study was to develop a double multiplex real-time PCR method for detection of the *C. difficile tcdA*, *tcdB*, and *cdtA* genes simultaneously in a single reaction. In the study, laboratory reference strains—including 14 *C. difficile* and 15 enteric pathogens—were used to validate the double multiplex real-time PCR assay. The PCR assay targeted and detected glucose-6-phosphate dehydrogenase (G6PDH; internal control) and *cdtA* genes in the first tube and *tcdA* and *tcdB* genes in the second tube, which were run simultaneously. The detection limit of the assay was 10^3 CFU/ml for *tcdA* and *cdtA*, and 10^2 CFU/ml for *tcdB*. The standard curves obtained with 10-fold serially-diluted bacterial suspensions ranged from10^1 to 10^7 CFU/ml. PCR efficiency of each assay was determined from the slopes of standard curves ($E=2^{[-1/slope]}$). The reaction efficiencies of real-time PCR for *tcdA*, *tcdB*, and *cdtA* were found to be 123%, 106%, and 85%, respectively. Correlation coefficients (*r*) for each of the reaction efficiencies were more than 0.99. None of the 15 non-clostridial control reference strains were detected with the multiplex real-time PCR assay, thus, yielding an analytic specificity of 100%. In conclusion, this double multiplex real-time PCR assay accurately detected *C. difficile tcdA*, *tcdB*, and *cdtA* in a single reaction.

PIII-6

DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR THE ASSESSMENT OF PROBIOTIC *LACTOBACILLUS BREVIS* CD2 STRAIN PERSISTENCE IN ORAL CAVITY

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Dental caries and periodontal disease occur in almost 95% of the population. Although the use of probiotics to improve oral health is still in its infancy, many clinical trials are ongoing in this field.

The aim of this study is to select the optimal conditions for quantification in the oral cavity of *Lactobacillus brevis* CD2, a probiotic strain contained in the commercially available dietary supplement Inersan® and to assess its persistency after oral administration. Different sets of primers for *L. brevis* detection have been tested in real-time PCR reactions to find optimal amplification efficiency and target specificity. Primers ability in the identification of *L. brevis* DNA was assessed in a pilot study conducted on 12 healthy volunteers clinically free of oral pathologies. The subjects took 3 tablets/day for 3 days and one tablet the 4th day.

Clinical samples (dorsal surface of tongue, first molar, vestibular fornix and saliva) were collected at baseline (before beginning the trial), at T0 (in the morning before the assumption of the last tablet) and 3, 6, and 9 hours after taking the last tablet. The quantities of *L. brevis* DNA were compared to the total number of bacteria present in the samples. The activity of the enzyme arginine deiminase, responsible for oral pH homeostasis (particularly abundant in the CD2 strain) was also assessed in the saliva samples before and after treatment by HPLC measurements. Enzymatic analyses revealed that arginine deiminase activity was higher at T0 and at 3 hours than at 6-9 hours.

We observed absence of tooth colonization by *L. brevis* at each time point, whereas its amount in the other oral sites was inversely correlated with elapsed time from the assumption. Persistence was maximal 3 hours after administration of tablets, but decreased more than one hundred times at 6 hours. However, the overnight recovery was significantly better than that obtained during the day, suggesting that probiotics administration before night could result in a better effectiveness.

MULTILOCUS VARIABLE NUMBER OF TANDEM REPEATS ANALYSIS OF *BACTEROIDES FRAGILIS* STRAINS

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Bacteroides fragilis is the most frequent anaerobic bacteria isolated from clinical specimens. Some studies were conducted to evaluate the genetic diversity of *B. fragilis* species. Here, we addressed this question, by using as a molecular tool for studying microbial diversity, the analysis of tandem repeats multilocus variable number (MLVA). MLVA is a prominent molecular tool for phylogenetic analysis and the resulting data will be helpful to establish clear clonal patterns in *B. fragilis* strains. Variable-number tandem repeats (VNTR) sequences were identified by comparison and analysis of the genome-sequenced NCTC9343 and YCH46 strains. After set the parameters that would define the tandem repeats of interest, a total of 17 VNTR loci were found in both strains and then 12 were selected. The single divergent locus between the two strains was also included, totalizing 13. We studied 23 clinical isolates, one obtained from environment and three isolated from human intestinal flora. *B. fragilis* (ATCC25285, ATCC43859, ATCC23745 and 638R) and *Bacteroides thetaiotaomicron* (ATCC29741) reference strains were also included. The partial results obtained from polymerase chain reaction (PCR) amplification of nine VNTR loci (BFR3, BFR5, BFR6, BFR10, BFR11, BFR12, BFR13, BFR14, BFR18) were analyzed and six loci (BFR3, BFR5, BFR6, BFR12, BFR14, BFR18) were polymorphic. Based on the distribution patterns of BFR3, it was possible to distinguish normal intestinal flora samples from other strains isolated from clinical specimens. The results of this study revealed remarkable genomic variability among the *B. fragilis* strains tested. However, further adjustments in the remaining VNTR loci would be necessary to appropriately incorporate this technique in our researches.

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PIII-8

AUTOMATED RIBOSOMAL INTERGENIC SPACER ANALYSIS AS A TOOL FOR SENSITIVE DETECTION AND RICHNESS ESTIMATION OF *CLOSTRIDIUM TYROBUTIRYCUM* IN COMPLEX FOOD MATRICES

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Clostridium tyrobutyricum is the main responsible of late blowing, a frequent alteration of semi-hard and hard cheese. It is due to butyric acid fermentation that leads to an anomalous organization of cheese loaf which prejudices structure of paste, cheese quality, and thus its commercial value. In response to the limits of conventional methods, advances in molecular tools now permit the detection of clostridia associated with late blowing, using faster PCR-based techniques.

This work aims at (i) detecting *C. tyrobutyricum* in raw milk and cheese by a new species-specific primer set through the use of the automated ribosomal intergenic spacer analysis (ARISA) method; (ii) considering the routine application of the method to monitor the presence of this bacterium that needs a fine-scale resolution. ARISA exploits the variability of the internal transcribed spacer (ITS) between the 16S and 23S genes to compare community structure across multiple samples and to estimate bacterial richness and diversity. It was chosen because it is more effective than other molecular tools in profiling both culturable and unculturable bacteria and because it permits to effectively study the spatial-temporal changes of populations in response to environmental factors.

The assay specific for *C. tyrobutyricum* was designed on strain LMG1285: sequencing of ITS amplicons allowed to detect a band specific of *C. tyrobutyricum,* used for primer design. After having verified its selectivity among *Clostridium* spp., the fluorescence-labelled fragment was resolved on an automated sequencer following the standard ARISA procedure. The sensitivity was also evaluated by using serial dilutions containing known amounts of genome equivalents ($3x10^6$-3).

The analysis of DNA from raw milks and Grana Padano P.D.O. cheeses collected seasonally in representative dairies is now in progress. It should also shade light on the temporal distribution of *C. tyrobutyricum* and on possible seasonal influences that could impact its concentration in milk and, consequently, in cheese.

This work was supported by MIPAAF (FILIGRANA Project).

MOLECULAR STRATEGY FOR CHARACTERIZATION OF A PLASMATIC FIBRONECTIN-BINDING PROTEIN IN *BACTEROIDES FRAGILIS*

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Bacteroides fragilis is the Gram-negative strictly anaerobic bacterium most frequently isolated from clinical infections. A number of factors can contribute to its virulence, including adhesins. Some of them are characterized and recognize and bind to the extracellular matrix components, such as, fibronectin (Fn). One of the molecules responsible for Fn-binding is an outer membrane protein previously described by our group, which belongs to the TonB dependent family of protein. Thus, the aim of this present work was to detect in *B. fragilis* strains the presence of the gene encoding for this protein by molecular characterization and also identify its localization in the bacterial surface. To perform this study, 20 *B. fragilis* strains isolated from patients with bacteremia and healthy subjects were selected. Initially, it was designed primers from the gene sequence deposited in a database (EMBL/GenBank). In all isolates tested by PCR, the presence of the gene responsible for the expression of the Tonb-dependent protein was confirmed. Then, the gene was cloned in a pET26b+ vector for construction of a recombinant protein-Adhesin-His tag (6xHis-Ads). The ligation product was transformed into competent cells of Rosetta cells for further purification and for adhesion assays. Concomitant protein expression, mutants from TonB gene was constructs, using the technique "tri-parental mating". Mutant and wild type strain have been used in an experimental animal model. An initial experiment under peritoneal macrophage survival showed that when the bacteria were coated with Fn, the bacterial survival was higher when compared to the bacteria not coated. The location of the Fn-binding proteins in the bacterial surface was conducted by transmission electron microscopy and immunostaining fluorescence microscopy. Further experiments are been performed to get a better understanding about the real role of this protein in the pathogenicity of *B. fragilis*.

PIII-10

COMPARATIVE EVALUATION OF BIOMERIEUX VITEK MS, BRUKER MICROFLEX MS, AND API AN FOR THE IDENTIFICATION OF CLINICALLY SIGNIFICANT ANAEROBES

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Objective: Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF)-based systems are replacing biochemical systems for routine identification (ID) of bacteria, hence we evaluated two MALDI-TOF MS and API AN systems for ID of clinical anaerobic pathogens.
Methods: A selection of 274 clinical isolates representing 4 genera and 14 species, obtained during a 7-month of routine laboratory processing of clinical specimens, were subjected to ID by the bioMerieux VITEK MS™ (VMS) and Bruker MICROFLEX MS™ (MMS) in parallel with conventional phenotypic API 20 AN (CPA) method. For VMS and MMS, isolates were tested in duplicates as single deposits of the same sets of isolates directly on MALDI-plate and for the API 20 AN, routine protocol was followed. ID interpretation was according to manufacturer's protocols; species were separated by a threshold of 50% matching peaks for VMS and ≥1.7=no reliable ID, 1.7-≤2.0=ID at genus level and ≥2.0=ID at species level for MMS. Discrepant results were resolved by 16S rRNA gene sequencing.
Results: VMS and MMS correctly ID all isolates to genus level. All systems correctly ID all species of the *Porphyronomas*, *Prevotella*, *Clostridium,* and *Peptostreptococcus*. bioMerieux VMS and API were in agreement in the ID of all (100%) *Bacteroides* spp., including all *B. fragilis* and *B. thetaiotaomicron* isolates, two of which Bruker MMS misidentified as *Malika spinosa* and *Propionibacterium acne*. The 2 discrepant results were resolved by 16S rRNA sequencing in favor of VMS and API AN. In addition, log scores of 8 isolates (2 *B. thetaiotaomicron*, 1 *B. fragilis*, 1 *P. bivia*, 1 *B. ovatus*, 1 *B. vulgatus* , 1 *C. difficile* , 1 *C. sporogenes* and 1 *P. assacharolyticus* were <1.7 in the Bruker MMS, i.e. unreliable ID. Likewise, another 24 scored 1.7-<2.0, meaning they could be validated only to genus level.
Conclusion: Both automated systems performed excellently well in terms of providing accurate ID 48-72h earlier than the CPA method. Both are user-friendly (bioMerieux VMS>Bruker MMS) and can be easily incorporated into routine diagnostic procedures.

A COMPARISON OF COMMERCIALLY PREPARED CULTURE MEDIA FOR THE ISOLATION OF ANAEROBIC BACTERIA

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Objective: The purpose of this study was to compare the recently launched AnaeroGRO™ pre-reduced anaerobic culture media (Hardy Diagnostics, Santa Maria, CA) to two existing products, BD™ (Sparks, MD) reducible media and Anaerobe Systems PRAS media (Morgan Hill, CA).
Method: Several anaerobic ATCC strains were evaluated on Brucella with Hemin & Vitamin K, PEA, BBE and LKV agar. ATCC strains included *B. fragilis* (ATCC 25285), *F. nucleatum* (ATCC 25586), *P. melaninogenica* (ATCC 25845), *C. perfringens* (ATCC 13124), *C. difficile* (ATCC 9689), and *P. anaerobius* (ATCC 27337). Three different lot numbers were parallel tested in triplicate for 3 consecutive days. A suspension of each strain was prepared in 3.0 ml of buffered gelatin diluent [(1)] to match a 3.0 McFarland standard. A final 1:100 dilution was prepared from this suspension. Each of the 4 plates was inoculated with 10ul of the final dilution and incubated anaerobically (48 hrs at 37° C). Plates were evaluated at 48 hrs for colony size, morphology, relative abundance or luxuriance and other growth characteristics. Plates were reincubated and read at 72 hrs for further pigment production. A total of 1,458 plates were compared in this study.
Results: Colonies on AnaeroGRO™ Brucella, BBE and LKV were larger for all strains tested. This feature was significant for some strains (*C. perfringens* and *C. difficile*). Anaerobe Systems PEA agar demonstrated slightly better performance in many cases. Double zone hemolysis of *C. perfringens* was easier to visualize on BD and Anaerobe Systems Brucella agar. Package inserts state that pigment is visible at 24 hrs on LKV, but that was not evident for all 3 brands in this study. At best, pigment was seen as a tan coloration of the colonies and extended incubation was required before pigment was clearly visible.
Conclusions: AnaeroGRO™ media demonstrated superior performance for all strains tested on Brucella, BBE, and LKV. Colonies were larger & more luxuriant, sometimes appreciably so. Larger, more robust colonies can offer a distinct advantage when working with mixed anaerobic cultures. Pigment production was slightly more intense on Anaerobe Systems LKV. BD and Anaerobe Systems media performed comparably overall.

PIII-12

MOLECULAR VARIATIONS IN SEQUENTIAL ISOLATES OF *CLOSTRIDIUM DIFFICILE* AND DELETIONS IN THE NEGATIVE REGULATOR

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Purpose: A) To determine the molecular epidemiology of *Clostridium difficile* from clinically infected patients and determine differences in sequential isolates, and **B)** to determine deletions in the first 425bp of the negative regulator *tcd*C, with a view to establish associations with clinical severity, recurrence and pathogenicity.
Methods: Stool samples were cultured from 339 patients who tested positive for *C. difficile* toxin by Toxin A+B ELISA and GDH combination testing between Aug 2010 and July 2011. Isolates were PCR ribotyped using a capillary sequencer. Samples from patients who had a previous episode were retrieved to compare changes in ribotype over time. Toxin genes *tcdA, tcdB* and variations in the first 425bp of the binding component of *tcdC* were determined by multiplex PCR.
Results: A) PCR ribotype 001 (13.6%) was the commonest, followed by ribotype 002 (10.6%), 005, 014, 015 (8.6% each), and 078 (5.6%). Multiple samples were available from 70 patients. Differences in ribotype were observed in 2.6%, 3.8%, 25%, and 34.5% isolates with time intervals between sampling of 0-20, 21-40, 41-60, and >60days respectively.
B) Of the 301 isolates that were available for toxin gene analysis, deletions were observed in 13.6% of them. A 19bp deletion was found in isolates of ribotype 002 (2.8% isolates) and ribotype 015 (46.4% isolates). A 39bp deletion was found in all 19 isolates of riboptype 078 and a 53bp deletion in all 8 isolates of ribotype 023.
Conclusion: Molecular epidemiology of *C. difficile* isolates is changing over time. Patients are likely to be infected by the same ribotype if an episode recurs within 40 days. One-year follow up of the patients after the first sample was received will determine association between molecular characteristics and severity, recurrence and mortality. Future investigations on toxin production are needed to throw light on differences in pathogenicity.

CHANGES OF GUT MICROBIOTA IN EXPERIMENTAL CLOSTRIDIAL INFECTION REVEALED BY MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY

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Clostridium difficile is the major leading cause of nosocomial antibiotic-associated diarrhea with increasing incidence and fatality. Since anti-microbial resistance is not clinically problematic, the risk of recurrent *C. difficile* infection (CDI) is increased in patients. Antibiotic treatment alters the composition of gut microbiota which may diminish protective immunity. Although alterations in gut microbial communities are widely expected to be associated with CDI disease states, the nature of these transitions are mostly unknown. Therefore, we established a mouse model of antibiotic-associated CDI to investigate how infection with *C. difficile* affects the spatial and temporal dynamics of the full gut microbial community. Bactria from feces and cecum contents were compared in the groups before antibiotic treatment, after antibiotic treatment and after CDI. We used the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) Biotyper system to identify microorganisms directly from cultured colonies. While the major components of the intestinal microbial flora, *Bacteroidetes*, *Firmicutes*, and *Lactobacilli*, were significantly decreased after antibiotic treatment, *Enterobacteriaceae* and *Enterococcus* were significantly increased after CDI. Our results demonstrate that antibiotics perturb the commensal microbiota that could disrupt the colonic immune homeostasis and set the stage for intestinal domination by bacteria associated with hospital-acquired infections. Understanding the complex microbial populations and determining their composition using MALDI-TOF MS approaches will increase our ability to identify patients at risk for developing clostridial infections and allow us to prevent CDI.

Anaerobe 2012

915 *POSTER SESSION III:* OTHER CLOSTRIDIAL INFECTIONS

Posters will be presented in Poster Session III
Sunday, July 1 915-1000.

THP-1 MACROPHAGE PHAGOCYTOSIS OF *CLOSTRIDIUM SORDELLII* IS SUPPRESSED BY PROSTAGLANDIN E2 AND INTRACELLULAR cAMP SIGNALING

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Purpose: To determine the extent to which prostaglandin E2 (PGE2) and its second messenger molecule cAMP regulate human macrophage (Mf) phagocytosis of *Clostridium sordellii*.
Background: The toxic shock-associated pathogen *C. sordellii* causes maternal infections following childbirth or abortion. The immuno-modulatory lipid molecule PGE2 is abundant in the pregnant uterus. Synthetic analogues of PGE2 inhibited rodent Mf phagocytosis of *C. sordellii*. We hypothesized that PGE2 would impair human Mf ingestion of *C. sordellii* via EP2 and EP4 receptors and downstream cAMP signaling networks.
Methods: PMA-differentiated human THP1 cells were treated with PGE2 (10μM, 1μM, 0.1μM), the EP2 agonist butaprost free acid (BFA; 10μM, 1μM), or the EP4 agonist L-902,688 (10μM, 1μM) x 15 min. cAMP analogues 8-Bromo-cAMP (nonspecific); 6-Bnz-cAMP (PKA-selective); and 8-pCPT (Epac- 1 selective) were tested at 1mM x 30 min. Cells were then challenged with FITC-labeled *C. sordellii* ATCC9714 and phagocytosis was quantified after 3 hr by fluorometry. Both cAMP levels and PKA activation were measured after PGE2 treatment.
Results: PGE2 increased cAMP, activated PKA, and inhibited phagocytosis of *C. sordellii*. 10μM and 1μM PGE2 significantly inhibited phagocytosis by 47.4 ± 9.0% and 50.1 ± 11.3%, respectively. Treatment with cAMP analogs demonstrated that 8-Bromo-cAMP, 6-Bnz-cAMP and 8-pCPT-cAMP all significantly inhibited phagocytosis of *C. sordellii* by 38%, 40%, and 28%, respectively. Treatment with EP2 or EP4 receptor agonists both inhibited phagocytosis, but EP4 was more potent than EP2.
Conclusions: PGE2 raises cAMP in THP-1 Mf, probably via EP2 and EP4 receptors, activating PKA and Epac-1 pathways to suppress *C. sordellii* phagocytosis. PGE2 present in the uterus might suppress innate immune responses against *C. sordellii.*

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PIII-15

ANAEROBIC MICROORGANISMS IN SAMPLES OF MODERN SOILS AND PALEOSOLS

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The preservation and remediation of soil is one of the priorities for environment protection. The soil samples collected at archeological sites which have not been exposed for a long time to anthropogenic pressure are the good experimental models to study of soil resilience.

The samples from the modern soils horizons and paleosols studied in the present work were taken from settlement Bolshe-Klyarinskoe (8-10th century BC) in Kamsko-Ustinsky area of Tatarstan and burial ground Murzichinsky II (6-8th century BC) in Alekseevsky area of Tatarstan, Russia.

The soil samples studied were identified as a leached chernozem that contains a significant amount of microbial biomass carbon (C). The highest value of microbial biomass C (1873±90mg C/kg) in the samples from upper horizons of buried and modern soils formed under hydromorphic conditions was determined. It has been established that the significant part of microbial biomass C in samples tested is formed by anaerobic bacteria. Anaerobic bacteria were much more detected in the buried soil samples taken from settlement Bolshe-Klyarinskoe and burial ground Murzichinsky II. Clostridia and denitrifying bacteria were most prevalent among the strictly and facultative anaerobic bacteria present in the samples analyzed, CFU/g - 10^6-10^7. This is three times more than that of the samples of background modern soil. The number of sulfate-reducing bacteria in the soil samples of burial ground Murzichinsky II formed under hydromorphic conditions was 18-50*10^6 CFU/g. This is an order of magnitude greater than for soils formed under automorphic conditions. The lowest number of sulfate-reducing bacteria in soil samples taken in burial ground Murzichinsky II has been obtained.

Microorganisms present in the buried soils as well as in the background modern soils contribute to the organic matter mineralization. In modern horizons, the high rates of decomposition processes can be explained by the constant inflow of organic matter while in the buried soils by the number and functional features of microorganisms and mainly anaerobic bacteria.

DESCRIPTIVE EPIDEMIOLOGY OF INFANT BOTULISM IN THE UNITED STATES: THE FIRST 35 YEARS

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Introduction: Infant botulism (IB) results from intestinal colonization and toxin production by *Clostridium botulinum*, *C. butyricum,* or *C. baratii* and was first recognized as a novel infectious disease in California in September 1976. The flaccid paralysis produced by absorbed botulinum toxin results in constipation, multiple bulbar palsies, generalized weakness, hypotonia, and occasionally, respiratory arrest. Suspect cases of IB may be treated with Human Botulism Immune Globulin (BabyBIG®), the public service orphan drug created, licensed, and distributed by CDPH.
Methods: Laboratory-confirmed cases were identified by public health surveillance and physicians' requests for BabyBIG®. An IB case was defined as a patient ≤1 yr old with symptoms consistent with the paralyzing action of botulinum toxin, in whom *C. botulinum* toxin or organisms were identified in feces.
Results: From 1976-2010, 2817 cases of IB (98.8% hospitalized, 0.6% outpatient, 0.6% sudden death) occurred in the US, representing ~65% of all US human botulism cases. 1241 (44.1%) were toxin type A, 1537 type B (54.6%), and 39 (1.4%) other. Median age at onset was 13 wks, and 50.0% were male. The 35-year incidence was 2.1 cases per 100,000 live-births, with a mean of 80 cases per year. Incidence was lower in Black and Native American infants than in White, Hispanic, or Asian infants. Cases occurred in all states including AK and HI; 1115 cases (40%) occurred in CA. States with higher incidence included CA, HI, MT, NM, OR, UT, and WA in the western US and DE, NJ, and PA in the eastern US. Mean age at onset was 8.6 wks for formula-fed vs 16.6 wks for breastfed patients ($P<0.01$, t test). Only 5% of cases were fed honey, a recognized source of botulinum spores.
Conclusions: IB occurs throughout the US, but is more prevalent in the western and mid-Atlantic regions. The lower incidence in Black and Native American infants suggests under-recognition of IB in these groups or the influence of other factors. The older age at onset for breastfed patients implies that breastfeeding may delay intestinal colonization by *C. botulinum*.

PIII-17

THE EFFECT OF *CLOSTRIDIUM PERFRINGENS* TYPE C AND ITS BETA TOXIN NULL MUTANT IN GOATS

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Clostridium perfringens type C is an important cause of enteritis and enterocolitis in several animal species including pigs, sheep, goats, horses, and humans. The disease is a classic enterotoxemia and the enteric lesions and associated systemic effects are thought to be caused primarily by beta toxin (CPB), one of two typing toxins produced by *C. perfringens* type C. This has been demonstrated recently by fulfilling molecular Koch's postulates in rabbits and mice. We present here an experimental study to fulfill these postulates in goats, a natural host of type C disease. Nine healthy male or female Anglo Nubian goat kids were inoculated with a virulent *C. perfringens* type C strain, an isogenic CPB null mutant or with a strain where the CPB mutation had been reversed (complement strain). Three goats inoculated with the wild-type strain presented abdominal pain, hemorrhagic diarrhea, necrotizing enterocolitis, pulmonary edema, hydropericardium, and death within 24 h of inoculation. Two goats inoculated with the CPB null mutants and two goats inoculated with sterile culture media (negative controls) remained clinically healthy during 24 h after inoculation and, no gross or histological abnormalities were observed in the tissues of any of them. Reversal of the null mutant restored its CPB production and virulence; 2 goats inoculated with this reversed mutant presented clinical and pathological changes similar to those observed in goats inoculated with the wild type strain, except that spontaneous death was not observed. These results indicate that CPB is both required and sufficient for *C. perfringens* type C to induce disease, supporting a key role for this toxin in type C disease pathogenesis.

PREVALENCE OF TOXIN PRODUCING *CLOSTRIDIUM* IN SOIL

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Purpose: Although *Clostridium* sp. are considered to be normally present in soil, the presence of neurotoxin producing *C. baratii* and *C. difficile* in soils in the United States has not been extensively studied. The aim of this study is to asses if neurotoxin producing *Clostridium* sp. and *C. difficile* can be detected in New York State soil samples.
Methods: Thirty-one soil samples were collected from public areas in 2 counties in New York State. Soil samples were inoculated in broth media. Aliquots of broth underwent nucleic acid extraction and were examined by PCR for the presence of *C. botulinum, C. perfringens*, *C. difficile*, *C. tetani,* and *C. baratii* toxin gene DNA. Aliquots of broth were also plated on selective agar to isolate *C. baratii* and *C. difficile*.
Results: PCR demonstrated the presence of *C. botulinum* type B DNA in 7samples (23%), and *C. botulinum* type E DNA in 4 samples (13%). Thirteen samples (42%) were positive for the presence of *C. tetani* DNA.

All soil samples were negative for the presence of *C. baratii, C. botulinum* toxin types A, C, and F, and epsilon toxin producing *C. perfringens* DNA. Alpha toxin genes of *C. perfringens* were demonstrated in all soil samples tested. Three samples (10%) were positive for the presence of *C. difficile* toxin B gene only, while 1 sample (3%) was positive for *C. difficile* toxin A and toxin B genes. Five soil samples were culture positive for *C. difficile*. A single isolate from 4 of the 5 culture positive soil samples was examined for the presence of toxin A and toxin B genes; all were negative for both genes. For the remaining soil sample, 11 isolates were tested for the presence of toxin A and toxin B genes. Five isolates were negative for toxin A and toxin B genes, while 6 isolates were positive for both toxin A and toxin B genes.
Conclusion: This study demonstrates the presence of toxin producing *C. difficile* in 13% of the soil samples studied. A mixture of toxin and non-toxin producing strains was found in one sample. All soil samples were negative for epsilon toxin-producing *C. perfringens*, as well as *C. baratii*. The presence of *C. tetani* and *C. botulinum* is similar to previously reported studies.

PIII-19

CLOSTRIDIUM PERFRINGENS TYPE E INFECTION IN A GOAT

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Clostridium perfringens is an important pathogen of enteric disease in domestic animals. It is classified into five toxinotypes based upon production of four major toxins. *C. perfringens* type E infection in domestic animals has been considered a rare occurrence, and there is no report of this type of infection in goats. Here, we report a first case of *C. perfringens* type E infection in a neonatal kid with a history of diarrhea and sudden death.

In August 2010, a 2-day-old kid with severe diarrhea and sudden death was submitted to the QIA. After necropsy, the tissues were routinely processed for histopathological examination including gram-stain. The intestinal content was incubated for 24h at 37°C in aerobic and anaerobic conditions. Multiplex PCR was performed to distinguish toxinotypes using the characteristic colonies. For detection of enterotoxin produced by the isolates, we used a PET-RPLA (DENKA SEIKEN, Japan). PCR was performed to detect rotavirus, coronavirus, border disease virus (BDV), and bovine viral diarrhea (BVD) virus for differential diagnosis.

Grossly, the intestine was distended with gas and watery intestinal contents. There were no specific lesions of the other organs except for remarkably thin intestinal wall. Histopathologically, the intestinal epithelium was exfoliated and lumen of the small intestine was filled with necrotic cell debris. A large number of gram-positive bacilli were attached to the mucosal layer, and some bacilli invaded into the lamina propria of the small intestine. Anaerobic culture of intestine confirmed a heavy growth of *C. perfringens*, which was determined by PCR analysis to be toxinotype E. The enterotoxin gene was also detected, but production of enterotoxin was not detected by PET-RPLA. Other pathogenic bacteria and viruses were not detected.

C. perfringens type E infection has been rarely reported, except reports of neonatal calves with hemorrhagic enteritis. To our knowledge, this is the first report of *C. perfringens* type E infection in a goat. More consideration should be given to type E-associated enteritis when planning for the health care of goats with neonatal diarrhea and sudden death.

SIALIDASES CONTRIBUTE TO DISEASES CAUSED BY *CLOSTRIDIUM PERFRINGENS* TYPE D STRAINS

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Clostridium perfringens type B and type D isolates, which produce the epsilon toxin (ETX), cause natural enterotoxemias or enteritis in livestock. ETX is listed as a CDC class B select toxin and also is very important for biodefense. Since most *C. perfringens* strains also express up to three different sialidases, this study focused on the contribution of those enzymes to type D pathogenesis. In type D isolate CN3718, *nanI* and *nanJ* encode secreted sialidases, while *nanH* encodes a cytoplasmic sialidase. Using CN3718, single *nanI*, *nanJ* and *nanH* null mutants, as well as a *nanI/nanJ* double null mutant and a triple sialidase null mutant were constructed. These mutants identified NanI as the major secreted sialidase of this strain and also showed synergistic effects of sialidase production on ETX expression. Cytotoxicity results showed that *nanI* can enhance epsilon-toxin-mediated cytotoxicity by promoting toxin binding. NanI can be activated by trypsin or chymotrypsin, but not NanJ and NanH. Furthermore, NanI also can be activated by mice intestinal fluid. Contact between BMC206 (an isogenic CN3718 *etx* null mutant that still produce sialidases) and certain mammalian cells (e.g., enterocyte-like Caco-2 cells) resulted in more rapid sialidase production, and this effect involved on increased transcription of *nanI* gene. BMC206 was also shown to adhere to enterocytes, but not other mammalian cells, and this effect was dependent upon sialidase, particularly NanI, expression. Collectively these *in vitro* findings suggest that, during type D disease origination in the intestines, trypsin may activate NanI, which (in turn) could contribute to intestinal colonization by *C. perfringens* type D isolates and also increase ETX action.

PIII-21

VIRULENCE FACTORS AND GENETIC DIVERSITY OF *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM CHICKENS WITH NECROTIC ENTERITIS

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Clostridium perfringens is considered the agent of necrotic enteritis (NE) in chickens, a disease that affects the poultry production worldwide. Toxins produced by *C. perfringens* are recognized as the main virulence factor in pathogenesis of NE; however other factors, can alter the host's immunity at the intestinal mucus and create conditions for bacterial colonization. In this study, toxinotyping, antimicrobial susceptibility, neuraminidase and hemagglutination (HA) production, as well as the genetic relationship among *C. perfringens* isolated from chickens, were determined. Twenty two strains were toxin type A and resistant to sulfaquinoxalin. Neuraminidase and HA production were observed, respectively, in 86% and in 27% of the strains. AP-PCR analysis showed a similarity of 83% among the strains, and they were grouped into a large cluster. Among them, four clusters showed strains with similar phenotypic characteristics. The values of MIC to antimicrobials were able to distinguish members of each cluster. Interestingly, one clone cluster (3IIB) was formed for two strains isolated from different animals, and it could represent a possible genetic pattern related to NE. In addition, the neuraminidase activities on chicken erythrocytes observed in the *C. perfringens* tested appear to be the first report, and it also may suggest a role in the NE pathogenesis. More studies detecting specific virulence markers associated with necrotic enteritis and the genetic diversity of *C. perfringens* from different sources are necessary.

THE MYSTERIES OF AZO DYE REDUCTION UNDER ANAEROBIC CONDITIONS

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Azo dyes are used widely in the textile, pharmaceutical, cosmetic, and food industries as colorants and are often sources of environmental pollution. There are many microorganisms that are able to reduce azo dyes by use of an azoreductase enzyme. It is through the reduction of the azo bonds of the dyes that carcinogenic metabolites are produced and are thus of concern to humans. The field of research on azoreductases is growing, but there is very little information available on azoreductases of strictly anaerobic bacteria. The azoreductase gene in *Clostridium perfringens* (AzoC) was recently identified, purified, and characterized by its enzymatic reactions with various dyes, and an important component for the optimal enzyme reaction was identified as the cofactors NADH and FAD. The purpose of this study was to investigate the varied reduction outcomes for different azo dyes under controlled and natural states (i.e. anaerobic/aerobic, pure/mixed state, etc).

Interesting outcomes occurred with different combinations of cofactors, non-azoreductase proteins, azo dyes, and anaerobic/aerobic environments. Each test sample contained the proper controls. The following dyes were tested, in order of increasing molecular weight: methyl red (mono-azo, unsulfonated), eriochrome black t (mono-azo, sulfonated), janus green (mono-azo, unsulfonated), tartrazine (mono-azo, sulfonated), congo red (di-azo, sulfonated), trypan blue (di-azo, sulfonated), direct blue 15 (di-azo, sulfonated), cibacron brilliant red 3b-a (mono-azo, sulfonated). Spectrophometric analysis of the test samples was conducted.

A strong cofactor effect was shown with some of the dyes under anaerobic and/or aerobic conditions. In addition, the presence of azoreductase or without azoreductase showed some variability in the final outcome. Interestingly, under aerobic conditions, the dye reduction with and without azoreductase was similar. Further chemical analysis of azo dye decolorization provided some insights into the mechanism of dye reduction. Our results suggest cofactors play an important role in azo bond reduction, and the participating molecules (protein, cofactors, and dye) may have competing roles.

PIII-23

CHARACTERIZATION OF PLASMIDS IN *CLOSTRIDIUM PERFRINGENS* TYPE A ASSOCIATED WITH NECROTIC ENTERITIS OF CHICKENS

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Clostridium perfringens type A isolates cause necrotic enteritis (NE), a common and important infection of broilers. Recently, NetB, a novel pore-forming toxin, has been described as an important factor for pathogenesis in NE. Our previous study showed that NE *C. perfringens* type A isolates harboured 3 to 5 different plasmids with considerable variation in size, ranging from 40 to 150 kb and that the *netB* gene is present in the pathogenicity locus (NELoc-1) located in an 80 kb plasmid. The CPB2 toxin (*cpb2*) gene is carried on a smaller plasmid (~60 kb) in most strains, together with the second pathogenicity locus, NELoc-3. Southern blotting showed that the *netB* gene was present in plasmids of slightly difference in sizes among a group of six strains and that one strain carried both *netB* and *cpb2* genes in the same plasmid. To understand further the virulence-associated plasmids found in NE isolates of *C. perfringens*, a pNetB and a pCpb2 plasmid were completely sequenced using the Roche 454 platform. pNetB and pCpb2 plasmids from NE isolates carried the *tcpA* and *tcpF* genes essential for conjugation.

To confirm that pNetB and pCpb2 plasmids are conjugative, these plasmids were marked with an antibiotic resistance gene and tested for their ability to mate to a laboratory strain (CW504). Both plasmids were confirmed to be conjugative.

The current study showed that 15 NE *C. perfringens* type A isolates and 5 isolated from healthy birds harboured 3 to 5 different plasmids with considerable variation in size, ranging from 40 to 150 kb. For most of the isolates, *netB* gene was located in NELoc-1, which was conserved among these isolates. There is remarkable variation in the plasmids found in strains of *C. perfringens* isolated with NE, probably because of recombination events between these related plasmids. For example, one isolate had two distinct plasmids, both of which contained NELoc-1 and NELoc-3 on the same plasmid. Another isolate had two distinct *cpb2* plasmids. Despite the plasmid variation, the NELoc-1 and NELoc-3 pathogenicity loci are well conserved in these plasmids.

HUMAN BOTULISM IMMUNE GLOBULIN FOR THE TREATMENT OF INFANT BOTULISM: THE FIRST EIGHT YEARS POST-LICENSURE

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Introduction: Infant botulism is the intestinal (infectious) toxemia in which swallowed spores of *Clostridium botulinum* or related species activate in the colon lumen and there produce botulinum toxin, which after absorption causes flaccid paralysis. Botulinum toxin has seven non-cross-neutralizable serotypes designated A-G. Human Botulism Immune Globulin (BIG-IV) neutralizes botulinum toxin types A-E. In October 2003, the US FDA licensed BIG-IV to the California Department of Public Health, its creator and developer, as the public service orphan drug BabyBIG® for treatment of infant botulism types A and B.
Methods: This report summarizes the October 2003-October 2011 distribution, safety, and efficacy data for BIG-IV. Case information was collected following BIG-IV treatment.
Results: In the US, a total of 651 eventually laboratory-confirmed patients residing in 43 states were treated within 1 week of hospital admission: 262 type A, 383 type B, 4 type Ba, and 2 type Bf. In the pivotal clinical trial of BIG-IV 1992-1997, mean length of hospital stay (LOS) for treated patients was 2.6 ± 1.5 wks vs. 5.7 ± 5.1 wks for untreated patients and did not differ significantly by toxin type (A or B) of illness. Mean LOS for BIG-IV-treated patients 2003-11 was also 2.2 ± 1.6 wks. No serious adverse events associated with BIG-IV treatment were reported. Aggregate hospital stay avoided 2003-11 through use of BIG-IV exceeded 35 years; aggregate hospital costs avoided totaled more than $50 million (2011 dollars). 2 type E and 4 type F patients were also treated with BIG-IV. Since July 2005, 27 laboratory-confirmed cases in 11 countries were also treated: 12 type A, 12 type B, 1 type Ba, 1 type E, and 1 type F. In the 15 years since open-label use of BIG-IV began, treatment of 986 US patients (397 type A, 581 type B, 5 type Ba, 3 type Bf) has resulted in more than 55 years of avoided hospital stay and more than $90 million (2011 dollars) of avoided hospital costs.
Conclusions: In the 8 years since licensure of human BIG-IV (BabyBIG®), its use to treat infant botulism patients has demonstrated its continued safety, efficacy, and US cost-effectiveness.

PIII-25

DIVERSITY OF CULTURABLE ANAEROBIC BACTERIAL ASSOCIATED TO THE MARINE SPONGE *APLYSINIA FULVA*

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It is estimated that over a million species of microorganisms live in the oceans of our planet. Due to their metabolic diversity, these microorganims exert a central role in the marine food chain and in the biogeochemical cycles of several elements. Molecular studies have recently shown that sponge-associated bacteria reflect the diversity found in the oceans, being associated with species from the three life domains. Marine sponges are filter feeders, sessile aquatic animals of the phylum Porifera. Studies demonstrate that 40-60% of their body mass can be formed by microorganisms living in a close mutualistic relationship. Some species are known as "bacteriosponges" with as much as 10^8 to 10^{10} bacteria per dry gram of weight. In Brazil, one of the most commonly found species is the *Aplysina fulva,* in which a rich bacterial community of at least 15 phyla was found, including anaerobic bacteria. These anaerobic bacteria are involved in several roles including the recycling of organic and inorganic components and bioaccumulation. In order to study anaerobic bacteria, new strategies for collection and the development of appropriate culture conditions is paramount in the description of new species. This work is aimed at the isolation, growth, and taxonomically description of anaerobic bacteria associated with *A. fulva*. First, 20 grams of live sponge were sonicated in 100ml of a pre-reduced buffer solution, serially diluted and platted in diverse culture media. All steps were performed in anaerobic environment with 10% CO_2 and 5% H_2. Forty different isolates based on colony morphology were obtained and submitted to aerobic and microaerophilic tests, in which 4 isolates proved to be strict anaerobes. Sequencing analysis of 16S ribossomal RNA revealed that one of the isolates was a *C. perfringens* and the remaining 3 seem to be undescribed species of the genus *Clostridium*. Our results unveil the opportunity of new studies and bioprospection of bioactive molecules produced by anaerobic bacteria associated to marine sponges.

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MOLECULAR DETECTION AND CHARACTERIZATION OF *CPB2* GENE AMONG *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM HEALTHY AND DISEASED CHICKENS

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Purpose: The aim of this study was investigation on *C. perfringens* isolates from poultry in Iran for the detection and characterization of beta2 toxin gene (*cpb2*).
Methods and Results: In this study, *C. perfringens* isolates from healthy and diseased poultry flocks from different parts of Iran were analyzed by PCR assay in order to determine the presence of all variant *cpb2* . The collection consisted of 36 *C. perfringens* isolates obtained from six Necrotic enteritis (NE)-positive flocks and 43 strains obtained from four NE-negative flocks was used for this study. The products of two positive *cpb2* PCR reactions were sequenced and comparison of these sequences to each other and to the *cpb2* sequences published in GenBank was made by multiple alignment and phylogenetic analysis. The present study represents the first investigation on *C. perfringens* isolates from poultry in Iran and as far as we know in other Asian countries for the detection of beta2 toxin gene and reports the highest percentage of *cpb2* positive isolates in both apparently healthy chickens (97.7%) and those associated with NE (94.4 %). The sequenced isolates belonged to atypical type.
Conclusion: We are reporting the highest percentage of *cpb2* gene presence in *C. perfringens* isolates in both healthy and diseased chickens. The sensitivity of detection of the various alleles of *cpb2* has been contributed to the primer sequences used for amplification. In the present study, we used primers capable of amplifying all known variants of *cpb2* sequences. Perhaps one of the reasons for the high prevalence of *cpb2* gene among isolates of this study was using appropriate primers in PCR reaction. We also found that the number of *cpb2*-harboring isolates was equally distributed among birds with NE and healthy ones. Surveillance of healthy chickens and chickens with NE has not revealed a direct correlation between occurrence of NE and presence of *cpb2* gene. Two Iranian isolates sequenced for *cpb2* were atypical.

PIII-27

EPSILON TOXIN IS ESSENTIAL FOR THE VIRULENCE OF *CLOSTRIDIUM PERFRINGENS* TYPE D-MEDIATED DISEASE IN SHEEP AND GOATS

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Clostridium perfringens type D causes disease in sheep, goats, and other ruminants. Type D isolates produce alpha and epsilon (ETX) toxins. However, some type D isolates can express up to five different toxins, raising questions about which toxins are most important for the virulence of these isolates. We evaluated the contribution of ETX to the virulence of *C. perfringens* type D in an intraduodenal challenge model in sheep and goats. Twenty-four healthy male or female cross bred sheep and 24 Anglo Nubian goat kids were inoculated with a virulent *C. perfringens* type D strain, an isogenic ETX null mutant, a complemented mutant that carried the wild-type *etx* gene on a shuttle plasmid, or with sterile, non-toxic culture medium (6 individuals of each species per inoculum). Five sheep and six goats inoculated with the wild-type D isolate developed acute clinical disease (sheep) and acute neurological disease and/or diarrhea (goats) followed by death. All the sheep and goats inoculated with this strain developed gross and histological changes which in sheep consisted of pulmonary edema, hydropericardium, and/or perivascular edema of the brain and in goats included necrotizing colitis, pulmonary edema, and/or hydropericardium. All the sheep and goats inoculated with the isogenic ETX toxin null mutants and those inoculated with sterile, non-toxic medium remained clinically healthy for at least 24 h after inoculation, and no gross or histological abnormalities were observed in the tissues of any animal. Complementation of the null mutant restored its virulence; all the goats and sheep inoculated with this complemented mutant presented clinical and pathological changes similar to those observed in the animals inoculated with the wild type strain. These results provide evidence that ETX is both required and sufficient for type D isolates to induce disease, supporting a key role for this toxin in type D disease pathogenesis.

A RETROSPECTIVE ANALYSIS ON CLINICAL BACKGROUND OF *CLOSTRIDIUM RAMOSUM* ISOLATED IN AICHI MEDICAL UNIVERSITY HOSPITAL

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We recently conducted a retrospective analysis on clinical background of *Clostridium ramosum* isolated and identified in Aichi Medical University Hospital during past 10 years from January 2001 to December 2010. All of 4 patients from whom *C. ramosum* was isolated were inpatients, and the frequency of isolation of *C. ramosum* among all clinical samples was 0.003% (4/121,264). The patients consisted of a man and 3 women, aged from 67 to 88 years. The underlying diseases were colonic cancer, rectal cancer, perforating peritonitis, and urinary tract infection. All patients had received surgery. The length of time from surgery to isolation of *C. ramosum* ranged from 0 to 13 days. The origins of the samples were drainage fluid, perianal pus, ascites fluid, and blood. Active infections were found in 3 cases, while unclear in a case. The clinical outcomes by antimicrobial chemotherapy were good (discharge) in 3 cases and dead in a case. Previous antimicrobial chemotherapy had been performed only in a case. Antimicrobial susceptibility test against *C. ramosum* isolated revealed susceptible to penicillins, cephems, and carbapenems, but intermediate or resistant to macrolides and lincomycins. Although infection caused by *C. ramosum* might be rare, this retrospective study would be useful for the detection of *C. ramosum* infection.

Anaerobe 2012

915 *POSTER SESSION III:* PROBIOTICS: MECHANISMS & HEALTH BENEFITS

Posters will be presented in Poster Session III
Sunday, July 1 915-1000.

PIII-29

BACTERIAL COUNTS FROM SIX OTC PROBIOTICS: ARE YOU GETTING WHAT YOU PAID FOR?

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The World Health Organization suggests that a probiotic consisting of a living organism, when given in adequate amounts, delivers a health benefit to the patient. There is concern that the bacterial colony counts present at the time of manufacture and listed on the package may not be reflective of the numbers present at the time of purchase and patient consumption. Consequently, this would hamper the effectiveness of the probiotic in the prevention of diseases such as antibiotic associated diarrhea and *C. difficile* infection. To investigate this issue, we performed a colony count study of six different commercially available probiotics.

Three separate samples each from a different store of six different probiotics (*Saccharomyces boulardii* [Florastor®], *Lactobacillus* GG [Culturelle®], *Bifidobacterium infantis* [Align®], 8 different species [VSL III®], *Lactobacillus acidophilus* [Lactinex®], and *Lactobacillus acidophilus* plus *Lactobacillus casei* [BioK+®] were purchased. We weighed out approximately 1 gram of power from each sample and placed it into a test tube and reconstituted it with sterile water to provide a 1:10 dilution. Lactinex which came as a tablet was crushed before testing. Serial 10-fold dilutions to obtain potential colony counts of up to 10^{-11} cfu/ml were prepared. A 0.1 ml sample from each tube was plated in duplicate onto blood agar plates (Hardy Diagnostics) to isolate the organisms stated in the packaging. Plates were incubated for 48 hours in an anaerobic chamber (except the *Saccharomyces* which was incubated aerobically), after which colony counts were performed to determine the viable cfu/gm.

Results showed that five of the six probiotics contained the stated amount of viable organisms. Lactinex had the lowest colony counts with 1.1-1.4 x 10 7 cfu/gm, but the packaging did not state an expected amount. The Florastor packaging also did not state an expected amount and was found to have 9.1 x 10^9 to 1.3 x 10^{10} cfu/gm. BioK+ had 5.6-6.1 x 10^9 cfu/gm.

Of the probiotics studied, most had adequate viable colony counts similar to those stated on the packaging.

INVESTIGATION OF PREBIOTIC CHARACTERISTICS OF INNOVATIVE FRUCTOOLIGOSACCHARIDES: PROBIOTICS METABOLIZATION AND ENTEROPATHOGENS INHIBITION

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Fructooligosaccharides (FOS) are well studied prebiotic. We aimed to investigate here the prebiotic characteristics of FOS synthesized from innovative matrix, which differ in their composition by their more varied intermediate polymers and specific additional carbohydrates.

Probiotic lactic acid bacteria belonging to the genus *Bifidobacteria*, *Lactobacillus*, *Lactococcus* and *Pediococcus* were used to evaluate the ability of these new FOS, to promote growth of beneficial strains. Raftilose®L95 was used as a commercialized FOS reference. Bacteria growths were monitored in medium with the tested carbohydrates as sole carbon source. Growth parameters showed that our FOS were metabolized by all probiotics, whereas only 3 strains used Raftilose®L95. HPLC analyses were conducted on supernatant to check the new FOS degree of polymerisation (DP) consumption. The shorter DP (DP2 and DP3) were preferentially consumed, while bifidobacteria were also able to metabolize DP4. Several enteropathogen growths were also checked when co-cultured with probiotics in a basal medium with the selected carbohydrates as sole carbon source. At the end of the incubation time, probiotics and pathogens were enumerated on selective agar. Among the tested pathogens, *Candida albicans* and *Clostridium difficile were strongly inhibited by some lactobacilli and bifidobacteria with new FOS. Listeria monocytogenes was also inhibited, but to a lesser degree. These inhibitions were significantly more important than those obtained with* Raftilose®L95.

We demonstrated here *in vitro* that these innovative FOS are more easily metabolized by probiotics and, when combined with probiotics, could help to inhibit more efficiently *C. albicans* and *C. difficile*, some major pathogens involved in antibiotic-associated diarrhoea, than one commercialized FOS. They are good candidates to promote gut beneficial bacteria and could be further evaluated for beneficial effects in gastrointestinal pathologies.

PIII-31

ANTIMICROBIAL AND ANTICYTOTOXIC CAPACITY OF A PROBIOTIC FORMULA OF *LACTOBACILLUS ACIDOPHILUS* CL1285 AND *L. CASEI* LBC80R AGAINST *CLOSTRIDIUM DIFFICILE* NAP1/027/BI

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Background and Objective: *Clostridium difficile* (CD) isolates of the molecular type NAP1/027/BI (NAP1) are the major cause of antibiotic-associated diarrhoea (AAD) and have been linked with severe diseases and hospital outbreaks worldwide. Probiotics are live microorganisms that, when administered in adequate amount, can confer a health-effect for the host. Recent clinical trials have demonstrated the capacity of a probiotic product named Bio-K+® (BIO-K+), containing *Lactobacillus acidophilus* CL1285® and *L. casei* LBC80R®, to reduce the incidence of AAD and *C. difficile* infections (CDI) in hospitalized adults receiving antibiotics. This study attempts to better understand the mechanisms through which BIO-K+ can neutralize *Clostridium difficile* NAP1 and prevents CDI.
Methods: The agar spot test was used to demonstrate the direct antagonism of the probiotic products (original strawberry, fruity, soy and brown rice) against NAP1 or nonhypervirulent strains of CD. The antimicrobial metabolites in the probiotic products were determined using cell-free supernatants (CFS) evaluated by well-diffusion assay. Prevention of the cytotoxic effect of CD by the probiotic product was evaluated on intestinal epithelial cell lines.
Results: Direct inhibition of a number of CD strains was obtained using the above mentioned probiotic products. The inhibition capacity of CFS was observed for all products with fermented soy, the strawberry and fruity products being the most active. Lactic acid production, differing between the probiotic products, is one of the antimicrobial metabolite while citric acid seems to have no impact. However, other antimicrobial molecules have yet to be purified and characterized. CFS obtained from BIO-K+ probiotic products were also able to reduce cytotoxicity of toxin A/B-containing CD broth. This effect seems to be linked to the presence of a protein.
Conclusion: This study demonstrated that BIO-K+ probiotics could inhibit the growth of CD and reduce the cytotoxicity mediated by CD toxin A/B *in vitro*, both factors being involved in CDI pathogenesis.

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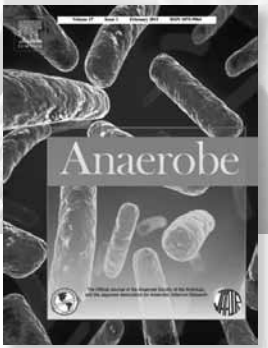

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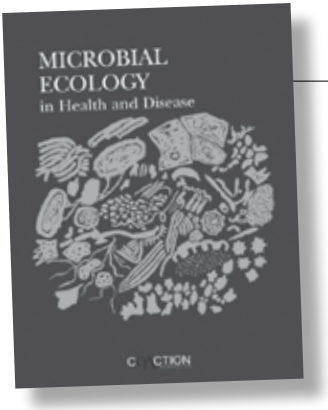

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