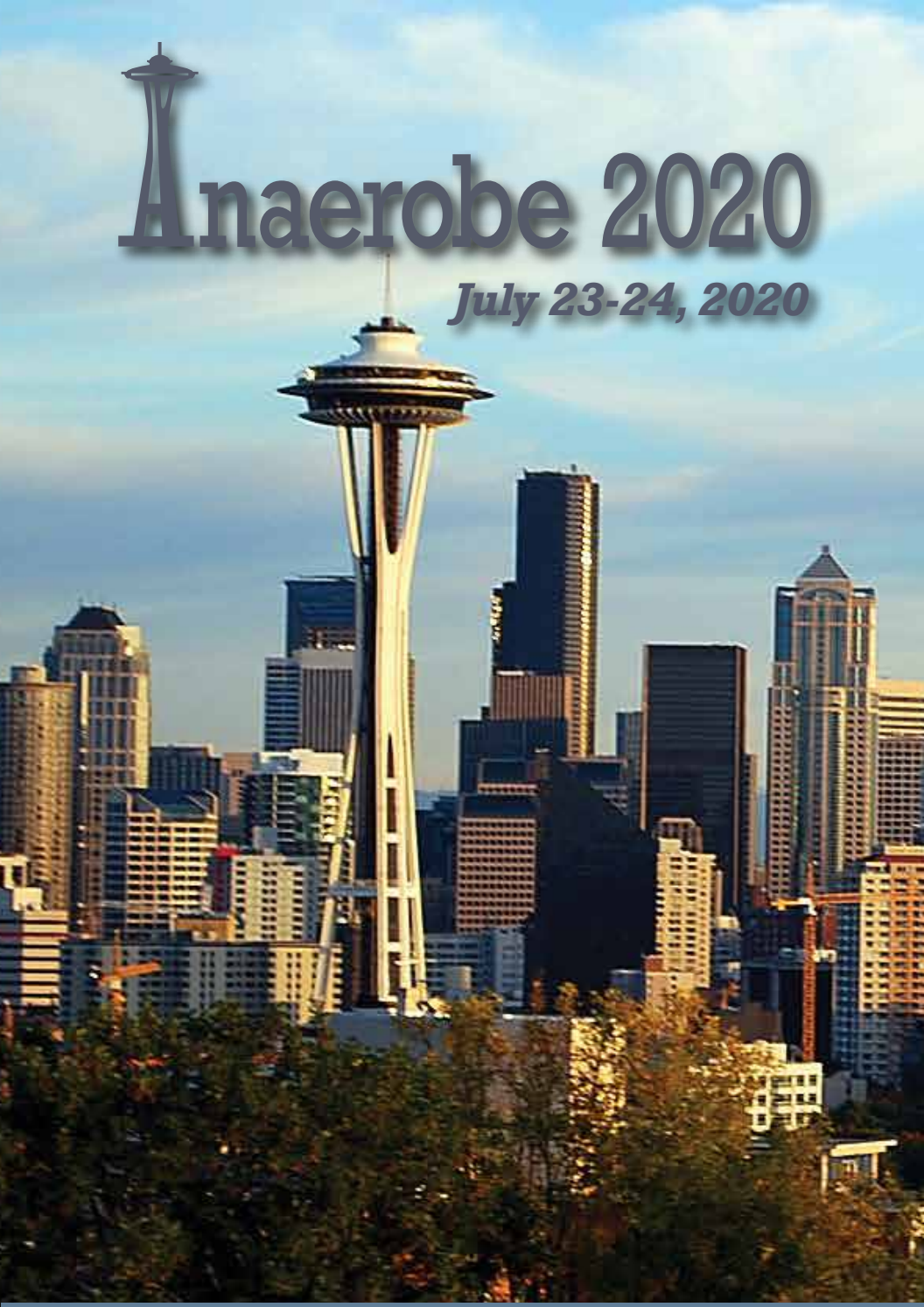
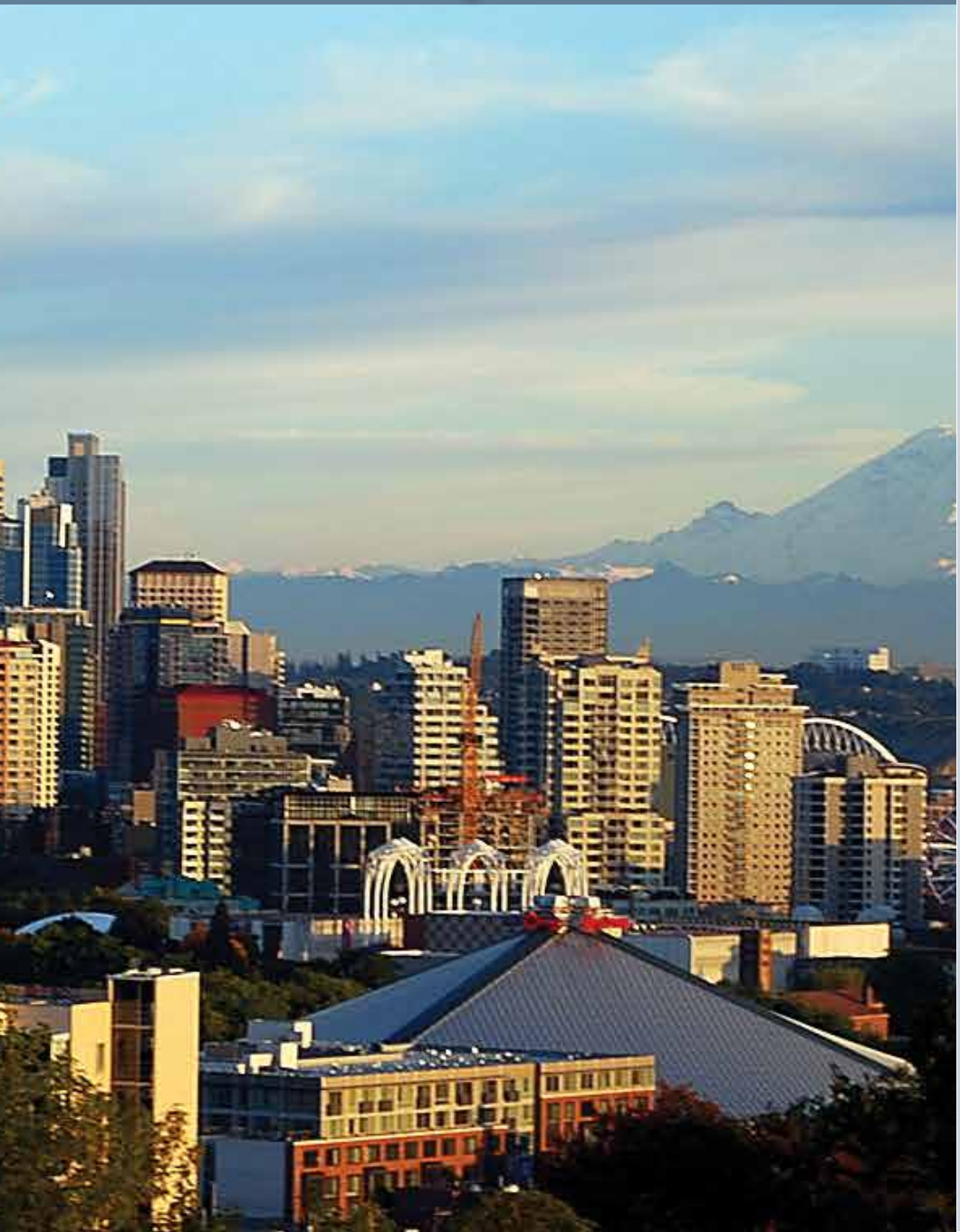


Anaerobe 2020

July 23-24, 2020

Program and Abstract Book

Virtual
The 15th Biennial Congress of the Anaerobe Society of the Americas



Program and Abstract Book

Anaerobe 2020
July 23-24, 2020

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July 23-24

Virtual
The 15th Biennial Congress of the Anaerobe Society of the Americas

Course Director

David N. Fredricks, M.D.
ASA President
Fred Hutchinson Cancer Research Center
Seattle, WA USA

Organizing Committee

David Aronoff, MD
ASA Past President
Vanderbilt University Medical Center
Nashville, TN USA

Susan Bartlett
Fred Hutchinson Cancer Research Center
Seattle, WA USA

Karen Carroll, MD
Johns Hopkins University
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Caroline A. Genco, PhD
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Medford, MA USA

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Hines VA Hospital
Maywood, IL USA

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Los Angeles, CA USA

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ASA Treasurer, Past President
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Los Angeles, CA USA

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New York, NY USA

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Birmingham, United Kingdom

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Birmingham, AL USA

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University of Washington
Seattle, WA USA

Elizabeth Nagy, MD, PhD
University of Szeged
Szeged, Hungary

Daniel Paredes-Sabja, PhD
Universidad Andrés Bello
Santiago, Chile

Cynthia Sears, MD
ASA Past President
Johns Hopkins University
Baltimore, MD USA

Aimee Shen, PhD
Tufts University
Medford, MA USA

Audrey N. Shuetz, MD
Mayo Clinic
Rochester, MN USA

Sujatha Srinivasan, PhD
Fred Hutchinson Cancer Research Center
Seattle, WA USA

Casey M. Theriot, PhD
North Carolina State University
Raleigh, NC USA

Vincent Young, MD, PhD
ASA Vice President
University of Michigan
Ann Arbor, MI USA

Francisco A. Uzal, D.V.M., PhD
University of California
Davis, CA USA

Dear Colleagues:
Welcome to ***Anaerobe 2020***, the 15th biennial Congress of the Anaerobe Society of the Americas! I am sorry that we will not be gathering in our usual format with oral scientific presentations, posters, and networking, but this has been an unusual time in our history that requires innovative approaches to connect scientifically, while maintaining physical distance. We identified early in the year that an in-person meeting would not be feasible, but it took some effort to convince our contractual partners that it would not be advisable to bring together large numbers of international professionals who have been treating patients with COVID-19 to a city that was experiencing the first outbreak in the U.S. Eventually, they all saw our point.

Instead, the Program Committee worked diligently to develop a plan for a virtual conference and whittled down the original program to a third, maintaining a mix of anaerobic-related topics presented by recognized authorities and emerging young investigators with academic, clinical, and industry representation. The response has been strong with over 450 participants from 33 countries. We are very appreciative that our scheduled Keynote Speaker, **Dr. Wendy Garrett** of Harvard Medical School, is able to participate to discuss her exploration of the relationship between anaerobic bacteria and colon cancer.

In order to recognize the fine research submitted for poster presentations, we are digitally publishing this Abstract Book, with a contact email for each abstract presenter to provide an opportunity for digital dialogue. ***Anaerobe 2020*** again illustrates the international interest in the field of anaerobic bacteriology with 160 abstracts representing the work of 610 scientists from 23 countries.

We would like to thank the members of the Organizing Committee and the Session Chairs for their assistance in formulating and adjusting what promises to be an exciting program. We also would like to thank our industry partners (Pfizer, Merck, Rebiotix, Acurx, and List Labs), the National Institutes of Health, the Burroughs Wellcome Fund, and the Fred Hutchinson Cancer Research Center for their support to make this Congress possible.

Special thanks goes to **Susan Bartlett** for helping to implement this virtual meeting format, and to **Dr. Ronald** and **Pamela Goldman** who planned for one format, adjusted for another, and continue to serve as organizers and advocates for our society.

Though this on-line Congress will not provide the interpersonal networking opportunities for which our biennial events are known, let ***Anaerobe 2020*** serve as a tantalizing preview for ***ANAEROBE 2022***, which we hope can return as an in-person conference, July 2022, in Seattle, WA USA.

David N. Fredricks, M.D.
President, Anaerobe Society of the Americas

About the Anaerobe Society

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

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

Anaerobe Society Congresses

This is the 15th biennial Anaerobe Society Congess.
Past Anaerobe Society sponsored programs were:

ANAEROBE 2018—Las Vegas, NV USA
ANAEROBE 2016—Nashville, TN USA
ANAEROBE 2014—Chicago, IL USA
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ANAEROBE 2008—Long Beach, CA USA
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ANAEROBE 2004—Annapolis, MD USA
ANAEROBE OLYMPIAD 2002—Park City, UT USA
2001: AN ANAEROBE ODYSSEY—Los Angeles, CA USA
ANAEROBE 2000—Manchester, England
ANAEROBE 1998—Buenas Aires, Argentina
ANAEROBE 1996—Chicago, IL USA
ANAEROBE 1994—Los Angeles, CA USA
ANAEROBE 1992—Los Angeles, CA USA

For Additional Information

About the Anaerobe Society or *Anaerobe 2020*

Web Site: www.anaerobe.org

Email: asa@anaerobe.org

Phone +310-216-9265

Fax: +310-216-9274

Goals & Objectives, Accreditation

Anaerobe 2020—the 15th 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, laboratory scientists, and industrial representatives.

Accreditation & Certificates of Attendance

Though planning for this Congress follows ACCME standards, this program is not accredited and no CME/CEUs will be issued. Certificates of Attendance may be requested.

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 xiv.

Evaluation Forms

Please complete the Evaluation Form at the end of the Congress.

Patrons

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

Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R13AI152307. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Keynote Speaker

Wendy S. Garrett, MD, PhD

Wendy Garrett is a Professor in the Departments of Immunology and Infectious Diseases and of Molecular Metabolism at the Harvard Chan School of Public Health, and also has a Professorship in the Departments of Medicine at Harvard Medical School. Dr. Garrett pursued an MD and PhD at Yale University. She completed a fellowship at the Dana-Farber Cancer Institute and postdoctoral training at Harvard University Medical School.

Dr. Garrett investigates host-microbiota interactions in health and disease. Her research team studies the interplay between the gastrointestinal immune system and the gut microbiota in health, inflammatory bowel disease (IBD), and colorectal cancer (CRC). The Garrett lab focuses on how the gut microbiota influence both innate and adaptive populations and the contribution of these cells to immune homeostasis and disease.

Dr. Garrett's team has identified specific species, pathways, and metabolites made by the microbiota that influence health and disease states. The lab also studies microbes and immune cells that are not only instrumental in potentiating carcinogenesis but are integral to intestinal homeostasis. The multi-faceted research approach includes meta'omics, microbiology, cellular immunology, biochemistry, cell biology, and cancer biology. The lab uses mouse models, human specimens, and primary and transformed mammalian cells and bacterial cells in their experiments in order to move facilely between large human data sets and *in vivo* and *in vitro* model systems with a core mission of determining basic biologic mechanism and applying the findings to precision medicine.

The Garrett lab is highly collaborative and works with many laboratories at institutions in the greater Boston area, in Harvard-affiliated hospitals and institutes, and at national and international research centers.

Garrett has received the following awards for her research: a Damon Runyon Foundation Fellowship, a Burroughs Wellcome Career in Medical Sciences Award, a V Foundation Scholar, a Cancer Research Institute Investigator Award, and a Searle Scholars Award. In 2020, she became a Fellow of the American Academy of Microbiology and the American Society of Clinical Investigation.

Presenters & Moderators

David M. Aronoff, M.D.
ASA Past President
University of Michigan
Ann Arbor, MI USA

Ken F. Blount, PhD
Rebiotix
Roseville, MN USA

Robert M. Centor, MD, MCAP
University of Alabama
Birmingham, AL USA

Jie (Angela) Chen
Johns Hopkins University
Baltimore, MD USA

Jon Clardy, PhD
Harvard University
Boston, MA USA

Laura M. Cox, PhD
Harvard Medical School
Boston, MA USA

Lawrence David, PhD
Duke University
Durham, NC USA

Esther Duperchy, PhD
Summit Therapeutics
United Kingdom

Craig D. Ellermeier, PhD
University of Iowa
Iowa City, IA USA

David N. Fredricks, M.D.
ASA President
Fred Hutchinson Cancer Center
University of Washington
Seattle, WA USA

Chris Ford, PhD
Seres Therapeutics
Cambridge, MA US

Caroline Ganobis
University of Guelph
Canada

Kevin W. Garey, PharmD
University of Houston
Houston, TX USA

Wendy S. Garrett, MD, PhD
Harvard University
Boston, MA USA

Caroline A. Genco, PhD
Tufts University
Boston, MA USA

Dale N. Gerding, M.D.
ASA Past President
Loyola University
Stritch School of Medicine
Maywood, IL USA

Yiping W. Han, PhD
Columbia University
New York, NY USA

Melissa Herbst-Kralovetz, PhD
University of Arizona
Phoenix, AZ USA

Andrew J. Hryckowian, PhD
University of Wisconsin
Madison, WI USA

Brendan J. Kelly, MD
University of Pennsylvania
Philadelphia, PA USA

Presenters & Moderators

Larry K. Kociolek, MD
Northwestern University
Chicago, IL USA

Zhenghui Li, PhD
Pfizer Vaccine Research
Pearl River, NY USA

Jennifer Linden, PhD
Weill Cornell Medicine
New York, NY USA

Sarah Lucas
University of Minnesota
Minneapolis, MN USA

Jeanne Marrazzo, MD, MPH
ASA Past President
University of Alabama
Birmingham, AL USA

Jeff S. McLean, PhD
University of Washington
Seattle, WA USA

Daniel Paredes-Sabja, PhD
Universidad Andrés Bello
Santiago, Chile

Jason Sahl, PhD
Northern Arizona University
Flagstaff, AZ USA

Prioty Sarwar
University of Pennsylvania
Philadelphia, PA USA

Audrey N. Schuetz, MD, MPH, D(ABBM)
Mayo Clinic
Rochester, MN USA

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

Mike Sheedlo, PhD
Vanderbilt University
Nashville, TN USA

Aimee Shen, PhD
Tufts University
Medford, MA USA

Andrew Skinner, MD
Loyola University Medical Center
Maywood, IL USA

Sujatha Srinivasan, PhD
Fred Hutchinson Cancer
Research Center
Seattle, WA USA

Rajani Thanissery
North Carolina State University
Raleigh, NC USA

Casey M. Theriot, PhD
North Carolina State University
Raleigh, NC USA

Alida Veloo, PhD
University of Groningen
The Netherlands

Vincent B. Young, MD., PhD
ASA Vice President
University of Michigan
Ann Arbor, MI USA

Thursday, July 23—*Times listed are Pacific Time Zone*

0645 **Congress Zoom Site Opens**

0700-0710 **Welcome and Overview:** *David Fredricks, MD*

0710-0815 **Session I: Keynote**
Moderator: David Fredricks, MD

Colon Cancer and the Gut Microbiota
Wendy S. Garrett, MD, PhD
Harvard University, Boston, MA USA

***Clostridioides difficile* Colonization Induces Colon Tumorigenesis in a Murine Model**
Jie (Angela) Chen
Johns Hopkins University, Baltimore, MD USA

0815-0915 **Session II: Clinical Infectious Disease: Anaerobe Infections**
Moderator: Jeanne Marrazzo, MD

Antimicrobial Resistance Mechanisms in Gram-Negative Anaerobic Bacteria
Alida Veloo, PhD
University of Groningen, The Netherlands

Anaerobic Mucin Degradation as a Bacterial Phenotype Associated with Chronic Rhinosinusitis
Sarah Lucas
University of Minnesota, Minneapolis, MN USA

Early Functional Metagenomic Changes Associated with the Targeted Spectrum Antibiotic, ACX-362E vs Oral Vancomycin in Healthy Volunteers
Kevin W. Garey, PharmD
University of Houston, Houston, TX USA

0915-0945 **Session III: Fusobacteria**
Moderator: Yiping W. Han, PhD

***Fusobacterium necrophorum* Pharyngitis and its Sequelae**
Robert M. Centor, MD, MCAP
University of Alabama, Birmingham, AL USA

0945-1015 **Break**

1015-1145 **Session IV: Bugs as Drugs: Engineered Microbial Communities & FMT**
Moderator: Vince Young, MD

Fecal Microbiota Transplant for Severe *C. difficile* Infection and During Critical Illness
Brendan J. Kelly, MD
University of Pennsylvania, Philadelphia, PA USA

The Next Generation of Fecal Microbiome Therapeutics
Ken F. Blount, PhD
Rebiotix, Roseville, MN USA

Treatment With SER-262, An Investigational Rationally-Designed Fermented Microbiome Therapeutic, Achieved a Lower Recurrence Rate in Subjects Treated for Primary *Clostridium difficile* Infection (PCDI) With Vancomycin (VAN) vs. Subjects Treated With Metronidazole (MET)
Chris Ford, PhD
Seres Therapeutics, Cambridge, MA USA

1145-1230 **Session V: The Oral Microbiome and Human Health**
Moderator: Jeff S. McLean, PhD

Oral Dysbiosis and Systemic Disease
Caroline A. Genco, PhD
Tufts University, Boston, MA USA

Molecular Basis of Cytotoxicity in Oropharyngeal Prevotella
Prioty Sarwar
University of Pennsylvania, Philadelphia, PA USA

1230-1300 **Break**

1300-1330 **Session VI: Functional Roles of Anaerobes in the Gastrointestinal Tract**
Moderator: Casey M. Theriot, PhD

Chemistry of Anaerobes: Plasmalogens
Jon Clardy, PhD
Harvard University, Boston, MA USA

1330-1400 **Session VII: Optimizing Anaerobic Cultivation**
Moderator: Sujatha Srinivasan, PhD

Microfluidic Cultivation of Human Gut Bacteria
Lawrence David, PhD
Duke University, Durham, NC USA

1400-1430 **Session VIII: Emerging Anaerobes and Disease Associations**
Moderator: David Fredricks

Identification of Emerging Anaerobes in the Clinical Microbiology Laboratory: MALDI-TOF MS and Sequence Analysis
Audrey N. Schuetz, MD, MPH, D(ABBM)
Mayo Clinic, Rochester, MN USA

Thursday, July 23—*Times listed are Pacific Time Zone*

1430-1445 **Break**

1445-1545 **Session IX: The Gut-Brain Axis: How Anaerobes Affect Neurological Development and Disease**
Moderator: Cynthia Sears, MD

The Intestinal Microbiota Influences Alzheimer's Disease Pathogenesis by Modulating Immunity and Amyloid-beta Processing in the Brain
Laura M. Cox, PhD
Harvard Medical School, Boston, MA USA

The Connection Between *C. perfringens* Epsilon Toxin and Multiple Sclerosis
Jennifer Linden, PhD
Weill Cornell Medicine, New York, NY USA

1545-1630 **Session X: Model Systems to Elucidate the Biology of Anaerobes**
Moderator: Aimee Shen, PhD

Leveraging Human 3-D Models and Omics to Study Host-Vaginal Microbiota Interactions
Melissa Herbst-Kralovetz, PhD
University of Arizona, Phoenix, AZ USA

Developing a Standard of the Murine Gut Microbiome for Murine Models
Caroline Ganobis
University of Guelph, Canada

1630 **First Day Concludes**

Friday, July 24—*Times listed are Pacific Time Zone*

0715 **Congress Resumes**

0730-0930 **Session XI: *Clostridioides difficile* I**
Moderators: Dale N. Gerding, MD and David Aronoff, MD

C. *difficile* Colonization in Infants and the Resulting Immune Response
Larry K. Kociolek, MD
Northwestern University, Chicago, IL USA

A One Health Perspective into the Prevalence of *Clostridioides difficile* (ST42) Across Clinical, Environmental, and Companion Animal Reservoirs Using Whole Genome Sequencing
Jason Sahl, PhD
Northern Arizona University, Flagstaff, AZ USA

Use of CRSIPRi to Study Gene Function in *C. difficile*
Craig D. Ellermeier, PhD
University of Iowa, Iowa City, IA USA

Structures and Functions of the *C. difficile* Toxins
Mike Sheedlo, PhD
Vanderbilt University, Nashville, TN USA

Changes in *Clostridiodes difficile* Molecular Epidemiology Coincide with Changes in Antibiotic Usage at One Hospital between 2005 and 2015
Andrew Skinner, MD
Loyola University Medical Center, Maywood, IL USA

0930-1000 **Break**

1000-1100 **Session XII: *Clostridioides difficile* II**
Moderator: Daniel Paredes-Sabja, PhD

Demonstration that a Bivalent Toxoid Vaccine Is Able to Induce Antibodies in Humans that Can Neutralize the Diversity of *Clostridioides difficile* Toxins TcdA and TcdB
Zhenghui Li, PhD
Pfizer Vaccine Research, Pearl River, NY USA

Ridinilazole Phase 2 Study: Minimal Impact on the Gut Microbiota and Bile Acid Metabolism is Associated With Low Rate of Recurrence of *Clostridiodes difficile* Infection
Esther Duperchy, PhD
Summit Therapeutics, Abingdon, United Kingdom

The Diet-Driven Metabolic Ecology of *Clostridium difficile* Infection
Andrew J. Hryckowian, PhD
University of Wisconsin, Madison, WI USA

Activity of Microbial Derived Secondary Bile Acid Iso-Lithocholate Against *Clostridioides Difficile* and Other Commensal Gut Microbes
Rajani Thanissery
North Carolina State University, Raleigh, NC USA

1100 **Congress Concludes**

Speaker Disclosurers

Ken F. Blount, PhD—Rebiotix (E)
Will discuss investigational products in development
Robert M. Centor, MD, MCAP—None
Jie (Angela) Chen—None
Jon Clardy, PhD—None
Laura M. Cox, PhD—Anaerobe Systems (C)
Lawrence David, PhD—None
Esther Duperchy, PhD—Summit Therapuetics (E, O)
Craig D. Ellermeier, PhD—None
Chris Ford, PhD—Seres Therapuetics (E, O)
Will discuss investigational products in development
Caroline Ganobis—None
Kevin W. Garey, PharmD—Acurx (G), Merck (G)
Wendy S. Garrett, MD, PhD—BioMx (C), Evelo Biosciences (S), Kintai (C), Leap Therapeutics (C), Sanofi-Genzyme (S), Tenza Therapuetics (C)
Caroline A. Genco, PhD—None
Melissa Herbst-Kralovetz, PhD—Lupin Pharamceuticals (C)
Andrew J. Hryckowian, PhD—Abbott Nutrition (G)
Brendan J. Kelly, MD—None
Larry K. Kociolek, MD—Infectious Diseases & Epidemiology Advising, LLC (O), Merck (G)
Zhenghui Li., PhD—Pfizer (E, O)
Will discuss investigational products in development
Jennifer Linden, PhD—None
Sarah Lucas—None
Jason Sahl, PhD—None
Prioty Ferheen Sarwar—None
Audrey N. Schuetz, MD, MPH, D(ABBM)—Pattern Bioscience (C)
Will discuss uses of MALDI-TOF MS
Mike Sheedlo, PhD—None
Andrew Skinner, MD—None
Rajani Thanissery—None
Alida Veloo, PhD—None

A=Advisory Board, C=Consultant, E=Employment, G=Grant, O=Ownership/Stock, P=Patent, R=Royalty, S=Speaker

Oral Abstract Contents

Oral abstracts are divided according to the Zoom webinar sessions. The table below identifies the pages pertaining to each session in the contents and among the abstracts.

Poster Abstract Contents

Thursday, July 23 **Keynote Session**

0710-0815 Session I: Keynote

* — Indicates Presenter

COLON CANCER AND THE GUT MICROBIOTA

Garrett, W.S.*
Harvard University, Boston, MA USA

Cancer has largely been considered a disease of genetic and environmental factors, however, increasing evidence has demonstrated a role for the microbiota (especially its anaerobic bacterial membership) in influencing tumor growth and spread, shaping anti-tumor immunity, and affecting therapeutic response. I will discuss both human data from meta'omics analyses and data from mechanistic studies in pre-clinical models that support that specific anaerobic bacteria act as potentiators or restraints of colonic tumorigenesis.

Email: wgarrett@hsph.harvard.edu

CLOSTRIDIOIDES DIFFICILE COLONIZATION INDUCES COLON TUMORIGENESIS IN A MURINE MODEL

Chen, J.;*[1] Wu, S.;[2] McMann, M.;[2] Lansiquot, C.;[3] Wu, X.;[2] White, J.;[4] Markham, N.O.;[5] Besharati, S.;[6] Anders, R.;[6] Tomkovich, S.;[7] Jobin, C.;[8] Sears, C.L.[1,2,3]
[1]Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD USA
[2]Department of Medicine, Division of Infectious Diseases
[3]Bloomberg-Kimmel Institute for Immunotherapy, Departments of Oncology and Medicine and the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD USA
[4]Resphera™ Biosciences, Baltimore, MD USA
[5]Vanderbilt University Medical Center, Nashville, TN USA
[6]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD USA
[7]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI USA
[8]Department of Infectious Diseases and Immunology, University of Florida, Gainesville, FL USA

Our recently laboratory studies identified that human colonic biofilms induced biofilms and colon tumors in germfree $Apc^{Min/+}$ $IL10^{-/-}$ and $Apc^{Min/+}$ mice, as well as specific-pathogen-free (SPF) $Apc^{Min/+}$ mice. Using 16S rRNA amplicon sequencing, we identified the colonization of *Clostridioides difficile* (*C. difficile*) in mice inoculated with a biofilm-positive human colon mucosal slurry, but not in mice inoculated with a biofilm-negative human mucosal slurry. These results presented an unexpected finding raising questions of whether persistent mucosal colonization of *C. difficile* contributes to biofilm formation or biofilm-associated tumorigenesis. Herein, we sought to test whether *C. difficile* persistent mucosal colonization induces colon tumorigenesis. To test this hypothesis, we utilized vancomycin and gentamicin pre-treatment to facilitate sustained and non-lethal *C. difficile* colonization for 12 weeks in the SPF $Apc^{Min/+}$ mice. We found that our human CRC-associated clinical isolate (CRC CI_m 2663) enhanced colon tumorigenesis at 12 weeks as compared to sham mice ($p= 0.0276$). The toxigenic *C. difficile* strain ATCC 9689 also displayed tumorigenic potential in a subgroup of mice, although the overall statistic was not significant ($p= 0.2243$). Persistent toxin B production in mouse colon correlated with tumor number ($p< 0.001$, $r^2= 0.8418$), suggesting a toxin B-dependent mechanism contributes to colon tumorigenesis in $Apc^{Min/+}$ mice. Of note, IL17, as a pro-oncogenic cytokine, was not differentially expressed in tumors or normal colon tissue from sham or infected groups. In contrast, IFNγ, an anti-tumorigenic cytokine, was increased in sham tumors but not in the tumors from *C. difficile* infected groups, which may indicate *C. difficile* suppresses anti-tumor immune responses. In summary, *C. difficile* colonization enhances colon tumorigenesis in $Apc^{Min/+}$ mice. This result may be dependent on both toxin B and immune mechanisms. Whole genomic comparisons, in progress, among *C. difficile* strains may provide supportive information to identify new tumorigenic virulence determinants.

Email: jchen212@jhmi.edu

Thursday, July 23 **Clinical Infections**

0815-0915 Session II: Clinical Infectious Disease: Anaerobe Infections

* — Indicates Presenter

ANTIMICROBIAL RESISTANCE MECHANISMS IN GRAM-NEGATIVE ANAEROBIC BACTERIA

Veloo, A.C.M.;* Boiten K.E.; Bathoorn D.; Rossen J.W.A.
University of Groningen, University Medical Center Groningen, Department of Medical Microbiology and Infection Prevention, Groningen, the Netherlands

Resistance to antibiotics can be caused by a variety of antibiotic resistance genes (ARGs) or other mechanisms, *e.g.* efflux pumps. For anaerobic bacteria, we have limited insight into the prevalence of known resistance genes. In addition, the exact mechanism responsible for the resistance has often not been identified.

Dutch *Bacteroides* (n=101) and *Prevotella* (n=99) isolates collected from 2015-2017 were in included. The prevalence of known ARGs was determined by PCR targeting the resistance genes *cfiA*, *cepA*, *cfxA*, *ermF*, *tetQ* and *nim*. Furthermore, isolates resistant to certain antibiotics, but in which no corresponding ARGs were encountered, were subjected to whole genome sequence (WGS) in order to reveal the possible resistance mechanism.

Within the *Prevotella* isolates, the presence of an ARG (*ermF* and *cfxA*) corresponded, in general, with the phenotypic clindamycin and amoxicillin resistance, respectively. This is in contrast to *Bacteroides* spp.: 95% of the tested isolates were resistant to amoxicillin, while 51.5% harbored an ARG (*cepA*, *cfxA* or *cfiA*) encoding a beta-lactamase. Presence of the *ermF* gene in *Bacteroides* isolates corresponded with clindamycin resistance. One *Bacteroides* isolate, susceptible for metronidazole, harbored a *nim* gene. Two *Prevotella* isolates were resistant to metronidazole of which one harbored a novel *nim* gene, *nimK*. The presence of this gene was revealed using WGS. In the other resistant isolate, no *nim* genes were encountered and are currently subjected to WGS.

In conclusion, our data suggests that a yet unknown beta-lactamase must be present causing resistance to amoxicillin within the genus *Bacteroides*. The use of WGS enabled us to reveal the presence of a novel *nim* gene in a *Prevotella* isolate. The impact of the presence of multiple ARGs in anaerobic bacteria and unknown resistance mechanisms, will be discussed.

Email: a.c.m.veloo@umcg.nl

ANAEROBIC MUCIN DEGRADATION AS A BACTERIAL PHENOTYPE ASSOCIATED WITH CHRONIC RHINOSINUSITIS

Lucas, S.K.;*[1] Itabiyi, R.A.;[2] Feddema, E.;[2] Boyer, H.C.;[2] Hunter, R.C.[1]
[1]Department of Microbiology & Immunology, University of Minnesota–Twin Cities, MN USA
[2]Department of Otolaryngology–Head and Neck Surgery, University of Minnesota–Twin Cities, MN USA

Chronic rhinosinusitis (CRS) describes a group of inflammatory disorders characterized by chronic sinonasal inflammation for which the etiology is incompletely understood. Although culture-based and genomic methods have determined that both healthy and chronically inflamed sinonasal mucosa are lined with distinct bacterial communities, the association with CRS remains unclear. Most antibiotic therapies for CRS are directed towards pathobiont *Staphylococcus aureus*, however, the phenotypes of CRS-associated bacterial consortia are not well understood. Moreover, research on the interbacterial interactions in the CRS microenvironment is limited. We hypothesized that bacterial consortia associated with CRS would have an anaerobic mucin-degrading phenotype, and that anaerobic mucin degradation could augment *S. aureus* growth *in vitro*. We used 16S rRNA gene sequencing to characterize bacterial communities of sinus mucus isolated during endoscopic sinus surgery in CRS and non-CRS patients. Sequences associated with anaerobic genera including *Fusobacterium*, *Prevotella*, *Peptostreptococcus*, and *Porphyromonas* were differentially abundant in CRS samples. Anaerobic enrichments of CRS sinus mucus in minimal medium with mucin (MMM) as the sole nutrient source yielded communities that were also characterized by genera *Prevotella*, *Fusobacterium*, and *Streptococcus* and have a cooperative mucin degradation phenotype. We demonstrate that *in vitro* aerobic growth of *S. aureus* on supernatants derived from CRS-enrichment cultures is enhanced compared to MMM alone, particularly when lactate is one of the major fermentation metabolites present. From these experiments, we conclude that there is a mucin-degrading anaerobic phenotype associated with bacterial communities found in the CRS sinonasal cavity. Furthermore, cooperative mucin-degradation by these communities can affect *S. aureus* physiology, potentially impacting virulence potential of this organism in the sinonasal niche.

Email: lucas156@umn.edu

EARLY FUNCTIONAL METAGENOMIC CHANGES ASSOCIATED WITH THE TARGETED SPECTRUM ANTIBIOTIC, ACX-362E VS ORAL VANCOMYCIN IN HEALTHY VOLUNTEERS

Garey, K.W.;*[1] Begum, K.;[1] Lancaster, C.;[1] Gonzales-Luna, A.J.;[1] Bui, D.;[1] Hu, M.;[1] Silverman, M.H.;[2] Alam, M.J.[1]
[1] University of Houston, Houston, TX USA
[2]Acurx Pharmaceuticals, White Plains, NY USA

Background: The mechanism by which a healthy microbiome loses colonization resistance after antibiotic use is poorly understood. Oral vancomycin has broad-spectrum activity against all major phyla of the human gut microbiome including *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*. ACX-362E, a DNA polymerase IIIC inhibitor in development for the treatment of *C. difficile* infections specifically targets low G+C Gram positive bacteria, including some *Firmicutes*. The purpose of this study was to compare early functional metagenomic changes associated with use of these antibiotics in healthy volunteers.

Methods: As part of the completed phase I clinical study of ACX-362E, stool samples were collected daily from volunteers given ten days of ACX-362E (300 or 450 mg given twice daily) or vancomycin (125 mg given four times daily). DNA was extracted from stool and sequenced using shotgun metagenomics (Illumina HiSeq). Early (day 3) and late (day 10) functional metagenomic changes were compared from baseline for volunteers given vancomycin and ACX-362E.

Results: Eighteen subjects (female: 33%) aged 30±8 years were enrolled. Baseline microbiota were similar between study groups and comprised of *Firmicutes* (62%), *Actinobacteria* (23%), and *Bacteroidetes* (11%). In subjects given vancomycin, host microbiota had been replaced by two distinct families, *Lactobacillaceae* or *Enterobacteriaceae,* by day 10 of dosing. At day 10, subjects given either dose of ACX-362E had replacement of *Firmicutes* by an expansion of existing baseline *Actinobacteria* microbiota, primarily *Bifidobacteriaceae*. Microbiota changes were assocaited with functional metagenomic changes in several functional classes at day 3 and day 10 of therapy.

Conclusions: Early microbiome changes after antibiotic therapy were associated with functional metagenomic changes. Use of the targeted-spectrum agent ACX-362E lead to distinct functional metagenomic profile compared to vancomycin.

Email: kgarey@central.uh.edu

Thursday, July 23 **Fusobacteria**

0915-0945 Session III: Fusobacteria

* — Indicates Presenter

FUSOBACTERIUM NECROPHORUM PHARYNGITIS AND ITS SEQUELAE

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

The Lemierre syndrome, a devastating infection, usually follows an episode of acute pharyngitis in an adolescent/young adult. The patients usually start with a sore throat. After around 5 days, they develop suppurative internal jugular thrombophlebitis with subsequent septic emboli. *Fusobacterium necrophorum*, a gram-negative anaerobe causes greater than 80% of this syndrome. Because this infection still has a mortality rate of approximately 5% and leads to long complex hospitalizations with a high percentage of prolonged intensive care, understanding its cause might lead to preventing this syndrome.

We performed a theoretical analysis that found this infection more dangerous, and thus more important than group A beta hemolytic streptococcal (GAS) pharyngitis for adolescents and young adults (approximately ages 15-30). This analysis postulates that the Lemierre syndrome is both more common and leads to more mortality and morbidity in this age range than does Acute Rheumatic Fever.

Because *Fusobacterium necrophorum* is an obligate gram-negative anaerobe, it does not grow on routine throat cultures. Only during this century have studies documented that this organism does cause endemic pharyngitis with the same signs and symptoms as GAS. In our college health center study, *Fusobacterium* pharyngitis occurred more commonly than Strep pharyngitis. Using the Centor score, the higher the score, the more likely the patient had a *Fusobacterium* infection.

Additionally, recent data show that this organism is also the most common cause of peritonsillar abscess in this same age group. There are good data that empiric antibiotics do decrease this suppurative complication.

So, we have a conundrum. We cannot easily diagnose *Fusobacterium necrophorum* pharyngitis, as routine cultures do not work (one can use special media, but this is not widely available) and PCR methods remain expensive. We, thus, make the case for empiric antibiotics for some patients in this age group despite no firm evidence that they would prevent suppurative complications. We hope that in the future, we have more data and better point of care testing.

Email: rcentor@uabmc.edu

Thursday, July 23 **Bugs as Drugs**

1015-1145 Session IV: Bugs as Drugs: Engineered Microbial Communities & FMT

* — Indicates Presenter

FECAL MICROBIOTA TRANSPLANT FOR SEVERE *C. DIFFICILE* INFECTION AND DURING CRITICAL ILLNESS

Kelly, B.J.*
University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA USA

The presentation will review clinical features that distinguish severe and severe-complicated (fulminant) *Clostridioides difficile* infection (CDI) from non-severe infection. Recent advances in understanding the pathogenesis of severe CDI and recent revisions to treatment recommendations for severe CDI will also be reviewed. Case series and clinical trials of fecal microbiota transplant (FMT) for the treatment of severe CDI will be examined, and key knowledge gaps will be discussed. Other applications of FMT in the critical care setting will also be briefly summarized.

Email: brendank@upennmedicine.upenn.edu

THE NEXT GENERATION OF FECAL MICROBIOME THERAPEUTICS

Blount, K.*
Rebiotix Inc, A Ferring Company, Roseville, MN USA

Recurrent *Clostridioides difficile* infections (rCDI) are associated with significant mortality, decreased quality-of-life, and substantial costs. Restoration of the composition and diversity of patients' intestinal microbiota is a promising strategy for reducing CDI recurrence, with fecal microbiota transplantation (FMT) procedures increasingly utilized. The lack of standardization of FMT process, product, and procedure has spurred the development toward standardized, quality-controlled, FDA-approvable microbiota-based therapeutics for rCDI. To achieve this aim and ensure access for patients, controlled trials of these investigational technologies are essential to demonstrate clear benefit and safety. Likewise, to fully understand how microbiota-based therapeutics may reduce rCDI, statistically rigorous microbiome and mechanistic characterization are needed.

Rebiotix, a Ferring company, is developing two standardized investigational products for reducing CDI recurrence. RBX2660 is a liquid suspension comprised of a broad, diverse consortia of living microbes, and RBX7455 is a room temperature-stable lyophilized formulation of living microbes administered by oral capsules. In three controlled Phase 2 clinical trials, RBX2660 reduced CDI recurrence with an acceptable safety profile and shifted participants' fecal microbiomes toward compositions associated with repression of *C. difficile* colonization. Concurrently, participants' fecal bile acid compositions rapidly resolved after RBX2660 treatment toward compositions thought to repress *C. difficile* growth. In a Phase 1 clinical trial, three dosing regimens of RBX7455 reduced CDI recurrence, were well tolerated, and induced fecal microbiome shifts toward compositions thought to repress *C. difficile* colonization.

In this presentation I will review the data from these clinical trials, along with the microbiome and metabolomic analyses performed on collect subject samples pre- and post-dosing. I will also discuss development of a Microbiome Health Index™—a promising microbiome-based biological marker that is consistent with clinical outcomes, to demonstrate the need for and value of a rigorous approach to assessing the full therapeutic potential of "bugs as drugs."

Email: ken.blount@ferring.com

TREATMENT WITH SER-262, AN INVESTIGATIONAL RATIONALLY-DESIGNED FERMENTED MICROBIOME THERAPEUTIC, ACHIEVED A LOWER RECURRENCE RATE IN SUBJECTS TREATED FOR PRIMARY *CLOSTRIDIUM DIFFICILE* INFECTION (PCDI) WITH VANCOMYCIN (VAN) VS. SUBJECTS TREATED WITH METRONIDAZOLE (MET)

Ford, C.;* Wang, E.; Bryant, J.; Nathan, R.; Hansen, V.; Pardi, D.; Brennan, R.; Pullman, J.; Bernardo, P.; Rogalin, H.; Litcofsky, K.; McGovern, B.; Trucksis, M.; Henn, M.
Seres Therapeutics, Cambridge, MA USA

Background: A low diversity microbiome is an underlying risk for CDI and CDI recurrence. This may account for a recurrence rate of approximately 25%, after antibiotic treatment of pCDI. SER-262 is an investigational consortium of 12 fermented Firmicute bacteria designed to restructure the microbiome and prevent recurrent CDI (rCDI) following pCDI.

Method: SERES-262-001 was a Phase 1b randomized placebo (PBO) -controlled single and multidose ascending dose study (1E4 to 1E8 SCFU). 96 subjects were enrolled across 8 cohorts (consisting of 12 subjects each, SER-262:PBO, 5:1). SER-262/PBO were dosed after MET or VAN for treatment of pCDI. The primary endpoints were safety and CDI recurrence up to 8 weeks after treatment. A secondary endpoint compared the engraftment of SER-262 strains following treatment with SER-262 or PBO.

Result: Safety of SER-262 was comparable to placebo. rCDI rates were similar overall between SER-262 and PBO treatment groups (18.8% and 12.5%, respectively) with no observed dose response across cohorts. However, there was a statistically significant reduction in rCDI rates in the SER-262 group treated with VAN for pCDI compared to those treated with MET (2/32 (6.3%) vs 13/48 (27.1%), respectively ($p = 0.02$, Fig. 1).

8 of 12 strains showed significant engraftment relative to PBO, with all 12 SER-262 strains detected in at least one subject at week 1. Engraftment of SER-262 strains was significantly greater in VAN treated subjects compared to MET subjects ($p<0.001$, Fig 2). Additionally, post-antibiotic baseline diversity was significantly lower in VAN vs MET subjects ($p<0.001$, Fig. 3).

Conclusion: SER-262 was safe and well-tolerated. Lower rates of CDI recurrence and higher rates of SER-262 engraftment were observed among subjects treated with VAN as compared to MET. Engraftment was likely facilitated by low baseline microbial diversity following VAN, which creates an ecologic niche for SER-262 species. rCDI rates in subjects receiving VAN and SER-262 (6.3%) was substantially lower than historical rates in subjects receiving VAN alone (20.6%).

Email: cford@serestherapeutics.com

Thursday, July 23 — **Oral Microbiome**

1145-1230 Session V: The Oral Microbiome and Human Health

* — Indicates Presenter

ORAL DYSBIOSIS AND SYSTEMIC DISEASE

Simas, A.M.;[1,2] Kramer, C.D.;[2] Genco, C.A.*[2,3]
[1]Graduate Program in Biochemical and Molecular Nutrition, Gerald J. and Dorothy R. Friedman School of Nutrition and Science Policy, Tufts University, Boston MA USA
[2]Department of Immunology, Tufts University School of Medicine, Boston MA USA
[3]Graduate School of Biomedical Sciences, Tufts University, Boston MA USA

Perturbation of the gut microbiome has been implicated in the development of non-alcoholic fatty liver disease (NAFLD) associated with consumption of a Western diet (WD). Recent evidence has also implicated dysbiosis of the oral microbiome in NAFLD progression. *Porphyromonas gingivalis* the etiological agent of oral inflammatory periodontal disease has been recently implicated in NAFLD and other cardiometabolic diseases, including atherosclerosis. We have previously established that *P. gingivalis* oral infection results in dysbiosis of both the oral and gut microbiome. However, whether changes to the gut and oral microbiota due to *P. gingivalis* oral infection influence the development of diet-induced cardiometabolic diseases has not been examined.

In the current study, we evaluated the impact of *P. gingivalis* oral infection on both the oral and gut microbiota of otherwise healthy mice and on dysbiosis of the gut microbiome and the progression of NAFLD in mice fed a WD. By first correlating the oral and cecal microbiota of *P. gingivalis*-infected mice fed a normal chow diet, we identified blooms of the Gram-positive genera *Barnesiella* and *Bacteroides* and imbalances of mucin-degrading bacteria. These disrupted community structures are predicted to have increased detrimental functional capacities, including increased flavonoid degradation, L-histidine fermentation, and imbalances of mucin degrading bacteria. These results validated the role *P. gingivalis* infection in dysbiosis of the gut microbiome, distal from the site of infection. We, then, contrasted the distinct and additive effects of *P. gingivalis* and WD feeding on NAFLD development, demonstrating that *P. gingivalis* infection induced sustained alterations of the gut microbiome composition and function in steatotic mice. Reduced abundance of short-chain fatty acid-producing microbiota was observed at both early and late timepoints post-infection. By examining the gut microbiota of *P. gingivalis*-infected mice fed different diets, we identified a common enrichment in the predicted microbiome capacity to produce cobalamin.

Collectively, our findings demonstrate that *P. gingivalis* infection produces persistent changes in the oral and gut microbiome composition and function that promotes hepatic steatosis and glucose intolerance suggesting a future avenue for investigation of the impact of the gut microbiota, diet, and *P. gingivalis* infection on cardiometabolic diseases.

Email: caroline.genco@tufts.edu

MOLECULAR BASIS OF CYTOTOXICITY IN OROPHARYNGEAL *PREVOTELLA*

Sarwar, P.;*[1] Carlson, R.;[2] Harasym, M.;[3] Planet, P.[1,4,5]
[1]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA USA
[2]Columbia University, College of Physicians & Surgeons, New York, NY USA
[3]Biology Department, The College of New Rochelle, New Rochelle, NY USA
[4]Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, PA USA
[5]Sackler Genomic Institute, American Museum of Natural History, New York, NY USA

Anaerobic bacteria are enriched and frequently isolated from the CF lung. While increased bacterial burden and repeated bacterial infections contribute to disease progression in CF patients, little is known about the role of anaerobic bacteria and their putative virulence factors in CF disease progression. The genus *Prevotella* is highly prevalent in CF patients and is often found in high abundance in the CF lung. To assess the pathogenic potential of *Prevotella*, we examined cytotoxicity using two strains of *Prevotella* (*P. melaninogenica* ATCC25845 and *P. scopos* 361B), and the *Prevotella*-encoded hemolysin, PhyA.

We found that *P. melaninogenica* and *P. scopos* both lyse red blood cells and cause cytotoxicity in the CF epithelial cell line, IB3-1, and its corrected counterpart, C38. However, *P. scopos* causes more robust hemolysis and lung epithelial cytotoxicity than *P. melaninogenica*. This suggests a possible link between blood cell lysis and lung epithelial cytotoxicity. One candidate virulence factor that could link these toxic phenotypes is the PhyA hemolysin that has been described previously. We find that PhyA expressed in *E. coli* can cause hemolysis and lung epithelial lifting and that both phenotype are abrogated by co-expression of the neighboring *phyZ* gene. Mass spectroscopy shows that when expressed along with the PhyZ protein in *E. coli*, PhyA loses lysine acetylation signatures. Furthermore, western blot analysis shows that when grown in media containing blood *P. melaninogenica* and *P. scopos* expresses more PhyA and PhyZ protein than when grown in media without blood.

Our data suggest that *P. melaninogenica* and *P. scopos*, common commensal organisms, may have pathogenic potential in CF through their cytolytic and hemolytic capabilities. The PhyA hemolysin is a potential virulence gene that could mediate host tissue destruction, and the activity of PhyA may be regulated, perhaps through posttranscriptional deacetylation, by PhyZ.

Email: priotyf@pennmedicine.upenn.edu

Thursday, July 23 **Anaerobes in the Gastrointestinal Tract**

1300-1330 **Session VI: Functional Role of Anaerobes in the Gastrintestinal Tract**

* — Indicates Presenter

CHEMISTRY OF ANAEROBES: PLASMALOGENS

Clardy, J.*
Harvard Medical School, Boston, MA USA

As part of a larger project exploring the small molecules produced by members of the gut microbiome, we encountered the class of lipids known as plasmalogens. These are glycerol-based lipids with a canonical head group, an acyl chain, and an enol ether, i.e. the usual fatty acid ester found at the sn-1 position is an enol ether. Mammalian plasmalogens are well known, and they are most frequently encountered in areas with high levels or reactive oxygen species (ROS). In the brain, for example, they form 70% of the glycerophospholipids in the myelin sheath covering dendrites and axons. The biosynthesis of mammalian plasmalogens is complicated, but well known, and genetic errors in the oxygen-dependent pathway are responsible for devastating developmental conditions.

Plasmalogens are also known from anaerobic bacteria, but their biosynthesis, distribution, and functions are poorly understood. We have used a knockout library in *Clostridium perfringens* to identify a putative biosynthetic gene that converts a sn-1 ester to a sn-1 enol ether – the formal product of a two-electron reduction. Evidence for this mechanism, metabolomic and bioinformatic analyses of its distribution, and preliminary studies on the possible role(s) of plasmalogens from anaerobic bacteria will be discussed.

Email: jon_clardy@hms.harvard.edu

Thursday, July 23 **Optimizing Anaerobic Cultivation**

1330-1400 Session VII: Optimizing Anaerobic Cultivation

* — Indicates Presenter

MICROFLUIDIC CULTIVATION OF HUMAN GUT BACTERIA

David, L.*
Duke University, Durham, NC USA

Culture and screening of gut bacteria enable testing of microbial function and therapeutic potential. However, the diversity of human gut microbial communities (microbiota) impedes comprehensive experimental studies of individual bacterial taxa. Here, I will present a platform that combines droplet microfluidics and high-throughput DNA sequencing to to separate and assay growth of microbiota members in picoliter droplets (MicDrop). MicDrop enabled us to cultivate a greater diversity of bacterial taxa than typical batch culture methods. We, then, used MicDrop to test whether individuals possess similar abundances of carbohydrate-degrading gut bacteria. We characterized carbohydrate utilization among dozens of gut bacterial taxa from distinct human stool samples. Our aggregate data across nine healthy stool donors revealed that all of the donors harbored gut bacterial species capable of degrading common dietary polysaccharides. However, the levels of richness and abundance of polysaccharide-degrading species relative to monosaccharide-consuming taxa differed by up to 2.6-fold and 24.7-fold, respectively. Additionally, our dataset suggested that gut bacterial taxa may be broadly categorized by whether they can grow on single or multiple polysaccharides, and we found that this lifestyle trait is correlated with how broadly bacterial taxa can be found across individuals. This demonstration shows that it is feasible to measure the function of hundreds of bacterial taxa across multiple fecal samples from different people, which may in turn enable future efforts to design microbiota-directed therapies and yield new insights into microbiota ecology and evolution.

Email: lawrence.david@duke.edu

Thursday, July 23 **Emerging Anaerobes**

1400-1430 Session VIII: Emerging Anaerobes and Disease Associations

* — Indicates Presenter

IDENTIFICATION OF EMERGING ANAEROBES IN THE CLINICAL MICROBIOLOGY LABORATORY: MALDI-TOF MS AND SEQUENCE ANALYSIS

Schuetz, A.N.*
Mayo Clinic, Rochester, MN USA

Identification of anaerobes in clinical microbiology laboratories has improved over recent years, due to advancements in matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and partial sequencing of the 16S rRNA gene. Many laboratories now commonly employ these methods for routine anaerobe identification. Alongside taxonomic updates of anaerobes, these methods have improved our knowledge of pathogen frequency and disease associations, leading to recognition of emerging species. Cases illustrating application of advanced anaerobe identification tools will be presented, and the challenges associated with utilization of these techniques will be highlighted. Novel adaptations of some of the more recent identification methods will also be discussed.

Email: schuetz.audrey@mayo.edu

Thursday, July 23 — **The Gut-Brain Axis**

1445-1545 Session IX: The Gut-Brain Axis: How Anaerobes Affect Neurological Development and Disease

* — Indicates Presenter

THE INTESTINAL MICROBIOTA INFLUENCES ALZHEIMER'S DISEASE PATHOGENESIS BY MODULATING IMMUNITY AND AMYLOID-BETA PROCESSING IN THE BRAIN

Cox, L.M.;*[1] Schafer, M.J.;[2] Vincentini, J.;[1] Wasen, C.;[1] Sohn, J.;[3] Weiner, H.L.;[1] Ginsberg, S.D.;[4] Blaser, M.J.[5]
[1]Ann Romney Center for Neurologic Diseases, Brigham & Women's Hospital, Harvard Medical School, Boston, MA USA
[2]Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN USA
[3]Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY USA
[4]NYU Neuroscience Institute, and Center for Dementia Research, Nathan Kline Institute, New York, NY USA
[5]Center for Advanced Biotechnology and Medicine, Rutgers University, New Brunswick, NJ USA

Alzheimer's disease (AD) affects an estimated 5.8 million Americans, and advanced age is the greatest risk factor. AD patients have been found to have altered intestinal microbiota and age-related changes in the microbiota contribute to immunologic and physiologic decline. We investigated the changes in gut microbiota in Tg2576 mice, a model of amyloid-beta deposition, and found that female Tg2576 mice have more substantial age-related microbiome changes compared to wildtype (WT) mice or compared to male mice, including an increase in *Bacteroides*. Elevated *Bacteroides* have been observed in aging humans and in AD patients compared to healthy controls. In the gut, we also found that Tg2576 female mice had an enhanced intestinal inflammatory transcriptional profile. We, then, found that administering a calorie-restricted diet controlled changes in the microbiome and in the intestine and prevented the accumulation of Ab plaque, which was specific to female mice. These results suggest that long-term calorie-restriction may alter the gut environment in a sex-specific manner and prevent the expansion of microbes that contribute to age-related cognitive decline. We, then, administered *Bacteroides* to another model of amyloidosis (the APP/PS1 mouse) and found that it increased Ab deposition, whereas the administration of the antibiotic metronidazole, which targets *Bacteroides*, decreased amyloid load. Both of these interventions altered peripheral immune responses and altered gene expression of Ab processing enzymes in the brain, which we identify as two potential mechanisms by which the microbiota can affect Alzheimer's disease.

Email: lcox@bwh.harvard.edu

THE CONNECTION BETWEEN *C. PERFRINGENS* EPSILON TOXIN AND MULTIPLE SCLEROSIS

Linden, J.*
Weill Cornell Medical College, New York, NY

C. perfringens epsilon toxin has been hypothesized to be an environmental trigger of Multiple Sclerosis (MS). MS is an immune-mediated disease of the central nervous system characterized by blood brain barrier permeability and demyelination. MS is believed to occur in genetically predisposed individuals exposed to an unknown environmental stimulant. Here, we present clinical and experimental data that indicates epsilon toxin may be a causative agent of MS. Serological, cellular, and fecal examination indicate that MS patients have increased exposure to epsilon toxin and the *C. perfringens* strains type B and D that produce it. *In vivo* and *in vitro* studies demonstrate that epsilon toxin recapitulates MS related pathology in the central nervous system, including blood brain barrier permeability and demyelination. Taken together, this data strongly supports the hypothesis that epsilon toxin may be the environmental trigger for initiating MS in humans.

Email: jel2049@med.cornell.edu

Thursday, July 23 — Biology of Anaerobes

1545-1630 Session X: Model Systems to Eluciate the Biology of Anaerobes

SX-1 Leveraging Human 3-D Models and Omics to Study Host-Vaginal Microbiota Interactions 32
Łaniewski, P.; Herbst-Kralovetz, M.M.*

SX-2 Developing a Standard of the Murine Gut Microbiome for Murine Models 33
Ganobis, C.;* Allen-Vercoe, E.

* — Indicates Presenter

LEVERAGING HUMAN 3-D MODELS AND OMICS TO STUDY HOST-VAGINAL MICROBIOTA INTERACTIONS

Łaniewski, P.;[1] Herbst-Kralovetz, M.M.*[1,2]
[1]Department of Basic Medical Sciences, College of Medicine-Phoenix, University of Arizona, Phoenix, AZ USA
[2]Department of Obstetrics and Gynecology, College of Medicine-Phoenix, University of Arizona, Phoenix, AZ USA

Specific bacteria residing in the female reproductive tract have been associated with increased risk of acquisition and transmission of sexually transmitted infections, pelvic inflammatory disease, miscarriage, preterm birth and most recently gynecologic cancer. The interrelationships between these bacteria and their functional impact on the cervicovaginal microenvironment that contributes to disease are poorly understood. Utilizing our three-dimensional (3-D) human epithelial cell culture models of the female reproductive tract, we evaluated bacterial vaginosis-associated bacteria (BVAB) species: *Gardnerella vaginalis, Prevotella bivia, Atopobium vaginae, Sneathia amnii, Sneathia sanguinegens*, and health-associated *Lactobacillus crispatus*. Immunoproteomics analysis for 28 targets and metabolomics analysis (418 metabolites) was performed. Hierarchical clustering analysis (HCA) of data revealed *A. vaginae, S. amnii,* and a polymicrobial cocktail distinctively modulate host immune and metabolism relative to *G. vaginalis, P. bivia, L. crispatus* and controls. *A. vaginae* exerted the greatest proinflammatory potential, significantly inducing pro-inflammatory mediators ($P<0.05$ to <0.0001) compared to controls. *A. vaginae* and *S. amnii* also exerted proinflammatory responses and increased levels of specific apoptosis-related proteins, heat shock proteins and growth factors ($P<0.05$ to <0.001) that relate to cellular stress. *G. vaginalis* infection resulted in a specific increase of specific matrix metalloproteinases and mucins ($P<0.01$ to <0.0001), whereas *P. bivia* decreased the levels of cytokeratins ($P<0.05$). Principal component analysis and HCA revealed that *A. vaginae*, *Sneathia* spp., and a polymicrobial cocktail exert more similar metabolic profiles and clustered separately from *G. vaginalis, P. bivia, L. crispatus* and uninfected controls. Random Forest analysis highlighted excellent predictive accuracy (93.75%) and identified biochemicals involved mostly in amino acid and nucleotide metabolism as top predictors of these BVAB and *Lactobacillus* colonization. Metabolomic analysis further extended our findings that *A. vaginae, S. amnii* and polymicrobial infection exhibit pathogenic properties. For example, dramatic changes in arginine and citrulline metabolism, which lead to inflammatory signaling via nitric oxide, were observed following infections with these BVAB. In conclusion, we identified vaginal bacteria with inflammatory, metabolic and epithelial barrier altering properties. Coupling our 3-D models with a panel of individual and polymicrobial cocktails and omics technology allowed us to provide novel insights into the mechanisms by which vaginal bacteria may contribute to reproductive and gynecologic sequelae.

Email: mherbst1@email.arizona.edu

DEVELOPING A STANDARD OF THE MURINE GUT MICROBIOME FOR MURINE MODELS

Ganobis, C.;* Allen-Vercoe, E.
University of Guelph, Guelph ON, Canada

In vitro and *in vivo* models are commonly used to study host-microbiome relationships. One of the most common *in vivo* models used in gastrointestinal and gut microbiome studies is the murine model. However, research findings from murine models can vary dramatically, and often are irreproducible and/or inconclusive. This is largely due to variances in gut microbial composition of mouse models caused by diet, genetics, husbandry, and environmental factors. If murine models are to be continued to be used as proxies for human disease, we must improve them by introducing a standardized, murine-derived gut microbiome of sufficient diversity.

This project aims to characterize and culture the murine gut microbiome, as it remains largely unexplored. To date, in this project, representative animals from seven mouse lines have been studied: five lines of C57BL/6 origin (IL10-/-, APC min-/- Msh2-/- , C57BL/6 N/N WT, C57BL/6J WT and fat-1), one of CD-1 origin (Nephrin-Y3F), and a wild-caught mouse (*Mus musculans)* from Algonquin Park, ON, Canada. Unsurprisingly, each animal tested displayed a unique gut microbial composition, with only three core microorganisms identified. While gut microbial diversity may differ, overall microbiome functionality may be conserved. To characterize the metabolic functionalities of murine-derived microbial communities, an *in* vitro bioreactor-based model of the mouse colonic environment has been developed and used to successfully support fecal and defined murine-derived microbial communities, as well as to derive defined murine gut microbial communities for further use.

Moving forward, our collection of murine-derived gut microbial defined communities will be introduced into germ free mice to compare resiliency and stability relative to mice inoculated with standardized but lower diversity ecosystems such as Altered Schaedler Flora. The ultimate objective of this work is to provide a uniform reference point for murine model work in the future, that takes into account the needs for experimental reproducibility, gut microbial diversity, as well as translatability to human health.

Email: ganobisc@uoguelph.ca

Friday, July 24 — *Clostridioides difficile* I

0730-0930 Session XI: *Clostridioides difficile* I

* — Indicates Presenter

C. DIFFICILE COLONIZATION IN INFANTS AND THE RESULTING IMMUNE RESPONSE

Kociolek, L.K.*
Northwestern University and the Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL USA

Infants are a unique population in terms of susceptibility to *Clostridioides difficile* infection (CDI). Despite frequent colonization with toxigenic (TCD) strains of *C. difficile* in the first year of life, symptomatic illness definitively attributed to *C. difficile* rarely, if ever, occurs in young infants. Although the reason for this in humans is unknown, animal models suggest age dependent differences in expression of receptors that bind *C. difficile* toxin A. Despite lack of CDI in infants, increases in seroprevalence of *C. difficile* anti-toxin antibodies throughout infancy and childhood has been observed. In a prospective cohort study of healthy infants, we investigated the immunologic consequences of TCD colonization during infancy. We collected stools from healthy infants throughout the first year of life to identify TCD colonization. We, then, collected serum at 9-12 months old to measure IgA, IgG, and IgM against TCD toxins A and B and neutralizing antibody (NAb) titers against toxin B. Among 32 infants, 12 (38%) were colonized with TCD at least 1 month prior to serology measurements. Infants colonized with TCD had significantly greater anti-toxin IgA and IgG against toxins A (p=0.02 for both) and B (p=0.009, p=0.008, respectively) compared to non-TCD-colonized infants. Five of 12 (42%) colonized infants had detectable NAb titers compared to zero non-TCD-colonized infants (p=0.02). These data suggest that previously observed rising seroprevalence during childhood may be related to TCD colonization. The protective effects of *C. difficile* anti-toxin seropositivity are unknown in children. However, population-level *C. difficile* surveillance suggests severe CDI is highly unusual in children. Further, there is a bimodal distribution of CDI incidence in childhood, with peaks in early childhood and late teenage years. The CDI incidence peak in early childhood may be related to a large proportion of susceptible toddlers, who did not experience TCD colonization in infancy, and a late teenage CDI incidence peak may be related to waning immunity. The extent and duration of protection against CDI later in life afforded by natural *C. difficile* immunization events requires further investigation, and these studies may guide *C. difficile* vaccination strategies in children.

Email: lkociolek@luriechildrens.org

A ONE HEALTH PERSPECTIVE INTO THE PREVALENCE OF *CLOSTRIDIOIDES DIFFICILE* (ST42) ACROSS CLINICAL, ENVIRONMENTAL, AND COMPANION ANIMAL RESERVOIRS USING WHOLE GENOME SEQUENCING

Williamson, C.; Stone, N.E.; Nunnally, A.E.; Hornstra, H.M.; Wagner, D.M.; Keim, P.; Sahl, J.W.*
Northern Arizona University, Flagstaff, AZ USA

Clostridioides difficile is a diarrheagenic pathogen that can cause symptoms ranging from mild disease to toxic megacolon and death. Several lineages of *C. difficile* have been frequently associated with *C. difficile* infection (CDI) in humans. One of these lineages is sequence type 42 (ST42) (ribotype 106), which has been associated with community-acquired CDI. Recently, ST42 has been identified as one of the most prevalent STs among adults in the United States. In this study, we investigated ST42 isolates from human clinical samples (n=33), environmental (soil, water) samples (n=24), and companion animals (n=15) in Flagstaff, Arizona. Our goals were to place these ST42 isolates into a global context of 415 ST42 genomes, to gain insight into potential sources of human infections, understand their prevalence in clinical samples, and to understand observed antimicrobial resistance. ST42 isolates from human clinical samples, environmental samples and companion animal samples were distributed throughout a core genome single nucleotide polymorphism phylogeny of the sequence type, and in some cases, isolates from human clinical samples are closely related to isolates from the environment or companion animals, which suggests that these non-healthcare-related reservoirs could be potential sources of human infections. *In silico* screening of ST42 genomes for antimicrobial resistance markers indicates that ST42 isolates from Flagstaff generally lack many of the antimicrobial resistance markers described in other lineages of *C. difficile*, suggesting that currently described antimicrobial resistance may not be a major factor in CDI cases associated with ST42 in northern Arizona. The presence of the ST42 lineage in multiple sample types in northern Arizona suggests that this lineage is an ecological generalist capable of survival in and transmission between multiple reservoirs. Understanding the ecology of this sequence type using whole genome sequencing, provides opportunities to better understand and mitigate transmission between humans and non-human reservoirs.

Email: jason.sahl@nau.edu

USING CRISPRI TO DISSECT LYSOZYME RESISTANCE IN *CLOSTRIDIOIDES DIFFICILE*

Ellermeier, C.D.*
University of Iowa, Iowa City, IA USA

Clostridioides difficile is a major cause of antibiotic-associated diarrhea and a leading cause of hospital-acquired infections. During an infection *C. difficile* encounters, various stresses including components of the innate immune system, such as lysozyme. Lysozyme acts by cleaving peptidoglycan at the ß-1,4-glycosidic bond between NAG and NAM residues leading to peptidoglycan degradation and cell lysis. *C. difficile* is highly resistant to lysozyme. We have found that the Extra-Cytoplasmic Function (ECF) σ factor, σ^V, plays an important role in lysozyme resistance. The activation of σ^V leads to transcription of lysozyme resistance genes. These include *pdaV*, a peptidoglycan deacetylase which removes the acetyl group from the N-acetylglucosamine residue, a common lysozyme resistance mechanism. Deletion of *pdaV* results in a modest 2-fold decrease in lysozyme resistance suggesting additional lysozyme resistance genes are required. In addition to *pdaV*, *C. difficile* encodes seven additional predicted polysaccharide deacetylases. We developed a CRISPRi system for use in *C. difficile* and performed a CRISPRi screen to identify additional deacetylases required for lysozyme resistance. We identified one, *pgdA*, that is required for lysozyme resistance. We show that deletion of *pgdA* or *pdaV* individually, results in a 2-fold reduction in lysozyme resistance. However in a double mutant, we observe a 1000-fold decrease in lysozyme resistance. Thus, the two deacetylases work to provide lysozyme resistance. Our data indicate that *C. difficile* has both intrinsic and inducible lysozyme resistances mechanisms and these systems work together to provide a high degree of lysozyme resistance in *C. difficile*.

Email: craig-ellermeier@uiowa.edu

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE *CLOSTRIDIOIDES DIFFICILE* TRANSFERASE TOXIN

Sheedlo, M.J.;*[1,2] Anderson, D.M.;[1,2] Jensen, J.L.;[1,2] Thomas, A.K.;[1] Lacy, D.B.[1,2]
[1]Department of Pathology, Microbiology, and Immunology. Vanderbilt University Medical Center, Nashville, TN USA
[2]The Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN USA

Clostridioides difficile are Gram-positive bacteria that exist in the environment as hardy spores. When the spores are ingested by humans, they make their way through the gastrointestinal tract and germinate. In situations where there have been large perturbations within the gut microbiota, such as those associated with antibiotic use, the bacteria can colonize and proliferate within the anaerobic environment of the colon. Some strains will begin to produce and secrete up to three toxins that target the colonic epithelium. Diarrhea is the primary symptom associated with the activity of these toxins, but the infection can develop to cause severe conditions such as pseudomembranous colitis and toxic megacolon, both of which are potentially deadly. The toxins produced by *C. difficile* can be separated into two distinct groups; the large *Clostridial* toxins (toxin A [TcdA] and toxin B [TcdB]) and the *Clostridioides difficile* Transferase toxin (CDT). The repertoire of toxins that is produced is heavily dependent upon the strain and as many as ~20% of clinical isolates have been found to produce all three. In these situations, the toxins are thought to act synergistically, potentially leading to a more severe form of the disease. The least well-studied of the toxins is CDT, a bipartite toxin that is comprised of two distinct polypeptides termed CDTa and CDTb. To gain insight into how CDT functions, we initiated cryo-electron microscopy studies with the aim of determining high resolution structures. We have determined five distinct structures of CDT to date. Four of these structures represent different states of the pore forming component, CDTb, and describe the transition of CDTb from the soluble state to the fully inserted pore. This analysis has led to the identification of important intermediates that exist during this process and has resulted in the identification of a new domain within CDTb that we have termed D3'. We have subsequently shown that the D3' domain is a glycan binding domain that is capable of interacting with simple sugars. As a part of our analysis, we have also proposed a potential surface that is used by CDTb to interact with the host cell receptor known as the lipolysis stimulated lipoprotein receptor (LSR). The fifth structure is that of the complete toxin, CDTb bound to CDTa. From this analysis, we observe one molecule of CDTa bound to one CDTb oligomer comprised of seven protomers. The orientation of binding places the N-terminus of CDTa in the middle of the oligomer and adjacent to important pore forming structures. We have shown that interactions of the CDTa N-terminus with CDTb likely drive CDTa translocation as removing portions of the N-terminus prevents cellular intoxication. Taken together, these data expand the current understanding of an understudied toxin and will serve as a starting point for delineating how CDT contributes to *C. difficile* pathogenesis.

Email: michael.j.sheedlo@vumc.org

CHANGES IN *CLOSTRIDIODES DIFFICILE* MOLECULAR EPIDEMIOLOGY COINCIDE WITH CHANGES IN ANTIBIOTIC USAGE AT ONE HOSPITAL BETWEEN 2005 AND 2015

Skinner, A.M.;*[1,2] Wieczorkiewicz, J.;[2,3] Cheknis, A.;[2] Stevens, V.W.;[4,5] Osmolski, J.;[3] Gerding, D.N.;[2] Johnson, S.[1,2]
[1]Loyola University Medical Center, Maywood, IL USA
[2]Hines VA Hospital, Hines, IL USA
[3]Midwestern University, Downers Grove, IL USA
[4]VA Salt Lake City Health Care System, Salt Lake City, UT USA
[5]University of Utah, Salt Lake City, UT USA

The molecular epidemiology of *Clostridiodes difficile* infection (CDI) in the U.S. has changed dramatically over the past decade with decreasing prevalence of the epidemic REA group BI (RT027) strain and emergence of new strains. We previously reported that BI was responsible for 72% of first CDI episodes at our hospital between 2005 & 2007. We repeated this survey between 2013 & 2015 at the same hospital and tracked antibiotic usage over both time periods.

REA strain typing and antibiotic susceptibility testing were performed on the recovered *C. difficile* isolates from 223 first episode CDI patients, between 2013-15, and compared to results from 143 patients with first CDI episodes, from 2005-2007. The antimicrobial days (AD) of selected antibiotics per 1,000 inpatient days was extracted from the VA corporate data warehouse for 2005-15.

REA group BI accounted for 42(19%) of first CDI episodes from 2013 - 2015, which represents a decrease of 73.8%($p < 0.001$) from 2005 – 2007. REA group strains DH & Y accounted for 27(12.1%) & 35(15.7%) of first CDI episodes in the later time period and were rarely seen in the earlier study. Fluroquinolone usage peaked in 2007 at 113 AD/1000 patient days and in 2015 had decreased to 55.9 AD/1000 patient days; moxifloxacin decreased from 21.8 to 0.8 AD/1000 patient days. Conversely, 3rd and 4th generation cephalosporins increased from 41.9 to 80.8 AD/1000 patient days; ceftriaxone increased from 18.6 to 46.1 AD/1000 patient days. Over this time period, *in vitro* moxifloxacin resistance decreased by 76.7%($p<0.001$), and ceftriaxone resistance increased by 90.7%($p<0.001$) among all *C. difficile* isolates tested.

Between 2005 and 2015, there was a significant decrease in CDI episodes related to REA group BI (RT027) infections with a corresponding increase in cases related to infection with REA groups DH (RT106) & Y(RT014/020). These changes corresponded to shifts in total inpatient usage of specific antibiotics; a decrease in fluoroquinolones and an increase in broad spectrum cephalosporins. Furthermore, these changes were reflected in a decrease in the *in vitro* resistance to moxifloxacin and an increase in the *in vitro* resistance to ceftriaxone among *C. difficile* strains at our hospital. Continued monitoring of *C. difficile* strain epidemiology and antibiotic usage patterns is warranted.

E-mail: andrew.skinner@lumc.edu

Friday, July 24 — *Clostridioides difficile* II

1000-1100 Session XII: *Clostridioides difficile* II

* — Indicates Presenter

DEMONSTRATION THAT A BIVALENT TOXOID VACCINE IS ABLE TO INDUCE ANTIBODIES IN HUMANS THAT CAN NEUTRALIZE THE DIVERSITY OF *CLOSTRIDIOIDES DIFFICILE* TOXINS TCDA AND TCDB

Li, Z.;* Lee, K.; Wellnitz, S.; Singer, C.; Rajyaguru, U.; Jones, H.; Anderson, A.S.; Liberator, P.
Vaccine Research and Development, Pfizer Inc., Pearl River, New York, NY USA

Clostridioides difficile is the most commonly recognized cause of infectious diarrhea in healthcare settings. Currently, there is no vaccine to prevent *C. difficile* infection (CDI). Two large toxins, TcdA and TcdB, are the primary virulence factors for CDI. Pfizer's investigational *C. difficile* vaccine, which consists of a mixture of genetically and chemically inactivated *C. difficile* toxoids A (TxdA) and B (TxdB), is currently in Phase 3 clinical trials. An understanding of the sequence diversity of the two toxins expressed by disease causing isolates is critical for the interpretation of the polyclonal immune response to the vaccine antigens.

In this study, we determined the whole genome sequence (WGS) of 478 *C difficile* isolates collected in 12 countries between 2004-2018 to probe toxin variant amino acid sequence diversity. A total of 44 unique TcdA variants and 37 unique TcdB variants were identified. Each of the TcdA variants shares at least 98% amino acid sequence identity with TcdA001, the variant used to construct the TxdA vaccine antigen. Sequence diversity among the TcdB variants is more substantial, ranging from 86.1% to >99% identity with TcdB001, the TxdB template. Phylogenomic analysis of the WGS data demonstrate that isolates grouped together based on ribotype (RT) or sequence type (ST) can code for multiple different toxin variants. A subset of sequence-diverse toxins was selected to measure the impact of toxin diversity on mammalian cell viability and the ability of polyclonal human immune sera to neutralize cytotoxicity. Serum samples from subjects immunized with Pfizer's investigational *C. difficile* vaccine were able to neutralize the cytotoxicity of toxins with sequences heterologous to the vaccine antigens, illustrating the broad coverage of the functional immune response to vaccination with these toxoid antigens.

Email: zhenghui.li@pfizer.com

RIDINILAZOLE PHASE 2 STUDY: MINIMAL IMPACT ON THE GUT MICROBIOTA AND BILE ACID METABOLISM IS ASSOCIATED WITH LOW RATE OF RECURRENCE OF *CLOSTRIDIODES DIFFICILE* INFECTION

Duperchy, E.;*[1] Qian, X.;[2] Yanagi, K.;[3] Kane, A.V.;[4] Alden, N.;[3] Lei, M.;[3] Snydman, D.R.;[4,5] Vickers, R.;[1] Roblin, D.;[1] Lee, K.;[3] Thorpe, C.M.[4,5]
[1]Summit Therapeutics, Abingdon, United Kingdom
[2]The Tufts Clinical and Translational Science Institute, Tufts University, Boston, MA USA
[3]Department of Chemical & Biological Engineering, Tufts University, Medford, MA USA
[4]Tufts Medical Center, Boston, MA USA
[5]Tufts University School of Medicine, Boston, MA USA

Background: *Clostridioides difficile* is a top 5 CDC urgent public health threat. *C. difficile* infection (CDI) generally requires perturbation of the gut microbiota, typically following antibiotic therapy. CDI is commonly treated with vancomycin (VAN) and metronidazole, which cause further collateral damage to the microbiota priming patients for recurrent infection, a central unmet medical need in CDI (~25% after a first episode). A healthy gut microbiota and its ability to metabolize human bile acids (BAs) is hypothesized to be a major mechanism of *C. difficile* colonisation resistance. Secreted BAs can promote *C. difficile* spore germination, while fully metabolized BAs can inhibit overgrowth. Ridinilazole (RDZ) is a novel targeted spectrum antibiotic under investigation to treat CDI and reduce recurrence (rCDI). Phase 2 results of RDZ and VAN therapy on rCDI, gut microbiota and bile acid (BA) composition are presented.

Materials/methods: A double-blind, randomized Phase 2 study randomized 100 patients 1:1 for 10 days RDZ or VAN treatment. Stool samples were collected from baseline up to 30 days post-end-of-treatment (EOT) and analyzed for microbiota and BA composition using 16S rRNA and LC-MS^2, respectively.

Results: The Phase 2 showed a ~60% lower rate of rCDI with RDZ (14%) compared to VAN (35%). RDZ treatment preserved the gut microbiota with no or minimal impact on the diversity and significant reduction in relative abundance at EOT of only a few taxa from the Firmicutes. In contrast, VAN showed reduction in alpha-diversity at EOT and dramatic reductions in four Firmicutes families (often to below detection) and in Bacteroidetes (>1000-fold). These were associated with an increase in Proteobacteria (>20-fold) including an expansion of numerous Enterobacteriaceae pathogens. RDZ also preserved the gut BA composition, whereas VAN showed a nearly 100-fold increase in the ratio of secreted to fully metabolized BAs at EOT compared to BSL, thus potentially priming for rCDI.

Conclusions: In Phase 2 RDZ showed ~60% reduction in rCDI compared to VAN. RDZ preserved the gut microbiota and BA composition providing a mechanistic rational to a higher *C. difficile* colonization resistance and lower rate of recurrence. RDZ is currently being evaluated in Phase 3 trials for superiority to VAN in sustained clinical response (cure and no recurrence at 30 days post-EOT).

Email: esther.duperchy@summitplc.com

THE DIET-DRIVEN METABOLIC ECOLOGY OF *CLOSTRIDIUM DIFFICILE* INFECTION

Hryckowian, A.J.;*[1,2,3] Van Treuren, W.;[3] Smits, S.A.;[3] Davis, N.M.;[3] Gardner, J.O.;[3] Bouley, D.M.;[4] Sonnenburg, J.L.[3]
[1]Department of Medicine, University of Wisconsin-Madison, Madison, WI USA
[2]Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI USA
[3]Stanford University School of Medicine, Department of Microbiology & Immunology, Stanford, CA USA
[4]Stanford University School of Medicine, Department of Comparative Medicine, Stanford, CA USA

Several enteric pathogens, including *Clostridium difficile* (*Cd*), leverage inflammation and dysbiosis to thrive in the gastrointestinal tract. Because host diet is one of the most powerful factors affecting the gastrointestinal ecosystem, we hypothesized that diet-driven changes to the gut microbiota would be key factors in dictating the outcome of *Cd* infection (CDI). Using a murine model of CDI, we show that mice fed diets deficient in the microbiota accessible carbohydrates (MACs) found in dietary fiber exhibit persistent infection. Conversely, mice fed a diet containing a complex mixture of MACs clear the pathogen within days. These distinct diets are accompanied by differences in the composition of the gut microbiota, the expression of *Cd* virulence factors, and the host inflammatory response, suggesting that diet- and pathogen-mediated inflammation together engender a *Cd*-permissive state. We further demonstrate that a diet containing a single, structurally defined, MAC type results in CDI clearance without increasing microbiota diversity. However, across the dietary conditions that exclude *Cd*, clearance is associated with a key subset of metabolic functions relating to MAC metabolism by the microbiota. Our data are consistent with the hypothesis that a MAC-deficient host diet reinforces a *Cd*-mediated inflammatory state that dictates a pathogen-supportive metabolic network during CDI. Our continued efforts will reveal mechanisms underlying the transitions between healthy and dysbiotic states, which can be exploited for a better understanding of the ecology of the gut microbiota and for the mitigation of CDI via targeted dietary intervention.

Email: hryckowian@wisc.edu

ACTIVITY OF MICROBIAL DERIVED SECONDARY BILE ACID ISO-LITHOCHOLATE AGAINST *CLOSTRIDIOIDES DIFFICILE* AND OTHER COMMENSAL GUT MICROBES

Thanissery, R.;* Vestal, A.G.; Theriot, C.M.
Department of Population Health and Pathobiology, NC State University College of Veterinary Medicine, Raleigh, NC USA

The loss of microbial derived secondary bile acids is associated with increasing susceptibility to *C. difficile* infection. Secondary bile acid isolithocholate (iLCA) inhibited seven clinically relevant strains of *C. difficile* at concentrations ranging from 0.027 – 0.054 mM, when compared to vancomycin at 2 μg/mL. Host derived primary bile acid chenodeoxycholate (CDCA) is biotransformed to lithocholate (LCA) via 7α-dehydroxylation by commensal *Clostridia*. Furthermore, LCA is detoxified to iLCA by bacterial epimerization of the 3–OH group through 3-oxo-intermediates using two enzymes 3α- and 3β-hydroxysteroid dehydrogenase (HSDH). iLCA is one of the most abundant bile acids in the healthy human gut with a concentration ranging from 0– 0.26 mM. We tested the MIC of CDCA and iLCA for *C. difficile* strain R20291 and a bacterial commensal panel including *C. scindens*, *C. hylemonae*, *C. hiranonis*, *B. thetaiotaomicron*, *L. acidophilus*, *L. gasseri*, *E. coli*, and *B. longum subsp infantis*. To investigate the mechanism of action (MOA) for iLCA, a time dependent kill assay was done at MIC and 10X MIC of iLCA compared to vancomycin (10 μg/mL). The MIC of CDCA (1 mM) was much higher than the MIC for iLCA (0.05 mM) for both R20291 and the commensal panel (>1 mM). At the concentrations tested, iLCA had no effect on the commensal panel, except some commensal *Clostridia.* Early log phase *C. difficile* cells were inhibited by iLCA at a concentration of 0.05 mM. At the same concentration, iLCA had bactericidal activity, causing a 3-log reduction of a late exponential phase population within 2 h of treatment, which was superior to vancomycin which resulted in a 1-log reduction. In conclusion, iLCA showed potent activity against *C. difficile* and spared most bacteria in the commensal panel. iLCA represents a novel compound that can target *C. difficile* while causing minimal damage to other gut commensals that provide colonization resistance against *C. difficile*. Future studies focusing on the iLCA mechanism of action, the enzymes that make iLCA, and efficacy studies in a mouse model of CDI are of interest.

Email: rthanis@ncsu.edu

Anaerobic Methodology

Anaerobic Methodology

OPTIMIZATION OF DNA EXTRACTION METHODS FOR LONG-READ WHOLE GENOME SEQUENCING OF *CLOSTRIDIOIDES DIFFICILE*

Adamczyk, M.;*[1,2] Paulick, A.;[2] Lutgring, J.D.;[2] Gargis, A.S.[2]
[1]Goldbelt C6, LLC, Chesapeake, VA USA
[2]Centers for Disease Control and Prevention, Atlanta, GA USA

Clostridioides difficile is among the most common causes of healthcare-associated infection in the United States. *C. difficile* has a dynamic genome with a high proportion of mobile elements. Use of short-read whole genome sequencing (WGS) alone results in fragmented *de novo* assemblies that can limit the ability to interpret genomic diversity. Comprehensive genetic characterization can best be achieved using both short- and long-read WGS data, which can generate finished genomes. Long-read sequencing platforms, e.g., PacBio, require large quantities (5-10 μg) of high molecular weight (HMW) DNA. Here we compare different DNA extraction methods and the resulting DNA concentrations and qualities to determine whether they can be used for long-read WGS.

Genomic DNA was extracted from cultured isolates (n=10) using the Promega Maxwell 16 Cell Low Elution Volume DNA Purification Kit and Maxwell 16 MDx Instrument, the MasterPure Complete DNA and RNA Purification Kit, and the QIAamp DNA Blood Mini Kit. Extractions were performed according to manufacturer's specifications, eluted in 50 µL volumes, with modified lysis conditions. For the MasterPure and QIAamp kits, addition of either an in-house lysis buffer, with or without lytic enzyme (lysozyme), or bead beating steps were assessed. DNA quantities were measured using the Quant-iT Broad-Range dsDNA Assay Kit. The quality of DNA was measured by optical density ratios, 260/280 and 260/230, and HMW was assessed using agarose gel electrophoresis. Differences in DNA concentration and quality were evident depending on the cell lysis and DNA extraction method. DNA extracted using the Maxwell kit had the lowest DNA concentrations (12-29 ng/μl). DNA yield was improved using the QIAamp (45-110 ng/μl) and MasterPure (119-285 ng/μl) kits with the addition of lysozyme to the in-house lysis buffer. Bead beating resulted in increased DNA shearing. The addition of in-house lysis buffer and lysozyme to the MasterPure kit was the only protocol that consistently produced HMW DNA with concentrations sufficient for PacBio WGS. Use of this extraction method for long-read WGS is essential for generating finished genomes to accurately assess genomic diversity.

Email: madamczyk@cdc.gov

BIOREACTORS: A VERSATILE TOOL FOR CHARACTERIZING MICROBIAL COMMUNITY DISRUPTION AND RECOVERY

Auchtung, J.M.*
University of Nebraska-Lincoln, Lincoln, NE USA

While disruptions in the gastrointestinal microbiome are associated with several diseases, it can be difficult to separate causation from correlation without tools that allow functional characterization of microbial communities. Continuous flow bioreactors are one tool that have been developed to study responses of microbial communities to perturbations under controlled conditions. To better understand the capacity of high-throughput continuous flow minibioreactor arrays to model dynamics of human fecal communities, we measured community stability and susceptibility to disruption following antibiotic treatment in complex communities cultured from fourteen healthy human fecal donors. We found that differences in microbial composition affected community stability *in vitro*, with enrichment of *Roseburia* and *Phascolarctobacterium* species associated with higher levels of community stability. Community composition also influenced the magnitude of disruption following treatment with different classes of antibiotics, although antibiotics that were broadly (vancomycin, imipenem, clindamycin, ciprofloxacin, and metronidazole) and minimally (sulfamethoxazole, cefaclor, azithromycin, fidaxomicin) disruptive were readily identified. The majority of complex communities cultured in vitro were resistant to colonization with *Clostridioides difficile*. Many antibiotics increased susceptibility to *C. difficile* colonization, though there was a wide range of outcomes depending upon community composition and antibiotic used. Somewhat surprisingly, there was little correlation between the overall magnitude of microbiome disruption following antibiotic treatment and susceptibility to *C. difficile* colonization. Ongoing studies are investigating how alterations in diet alter community stability and identifying key similarities and differences between *in vitro* and *in vivo* models of *C. difficile* colonization resistance.

Email: jauchtung2@unl.edu

MICROBIAL CULTUROMICS, A TOOL FOR CULTURING MICROBIOME ANAEROBES

Dione, N.;* Bilen, M.; Pereira, N.; Fischbach, M.
Bioengineering Department, Stanford Uuniversity, Stanford, CA USA

Anaerobic bacteria play a key role in clinical microbiology, causing a wide array of infectious and metabolic diseases. Since they are the major component of the human gut microbiota, scientists started investigating how the presence, absence, and proportion of these bacteria can lead to the development of diseases, symptoms and response toward some of the current treatments. For examples, studies showed its role in obesity, diabetes, inflammatory bowel disease (IBD), and cancer.

Recently, fecal microbiota transplantation, by its ability to cure patients infected by *Clostridium difficile,* highlighted the importance and the need to further study and isolate the bacterial components of the gut microbiota. However, the mechanisms of the reversion of this dysbiosis have not been clearly elucidated, since only part of the bacterial community in stool is known with few being isolated by culture.

In order to elucidate the mechanisms by which anaerobes are involved in all these processes, culturing the bacterial community from stool sample can generate biological material for further analysis.

Recently, a culturing approach using multiple culture conditions, matrix-assisted laser desorption/ionization–time of flight and 16S rRNA sequencing called Microbial Culturomics has been introduced. Culturomics is the method to date that has isolated the largest number of anaerobic bacteria from the digestive tract, including new species, and has significantly expanded the microbiome's anaerobic repertoire.

In this work, through the use of culturomics, we developed specific strategies that permit to isolate and identify a significant number of anaerobes from stool samples at a reduced time. This culture approach opens up interesting prospects towards the identification and characterization of commensal bacteria from the gut microbiota and would allow us to study the microbial interaction, and build microbial complexes for the treatment of certain chronic diseases.

Email: ndione@stanford.edu

AN INNOVATIVE *IN VITRO* ROTATORY CULTURE SYSTEM TO ASSESS THE EFFECT OF ANTIBACTERIAL ACTIVITY OF ALOIN AGAINST INTESTINAL COMMENSAL BACTERIA

Gokulan, K.;* Kolluru, P.; Cerniglia, C.E.; Khare, S.
Division of Microbiology, National Center for Toxicological Research, USFDA, Jefferson, AR USA

There are contradictory reports on the safety of aloe vera products that are widely used as ingredients in a variety of food, cosmetic and pharmaceutical products. *In vivo* studies have shown that ingestion of aloe leaf/purified aloin cause intestinal goblet cell hyperplasia, and malignancy. Here, we used non-animal model to determine the effects of aloin on intestinal microbiota. First, we used pure bacterial culture to evaluate antibacterial properties of aloin. The minimum inhibitory concentration ranged from 1 to 4 mg/ml and was also dependent on the anerobic or aerobic environment. Next, we tested the effects of aloin on the bacterial population present in the colon content. Current *in vitro* models are either static or have limited interaction of test agent with fecal microbiota. To overcome this pitfall and to mimic peristaltic movements of the gastrointestinal tract (GIT), we designed an innovative *in vitro* culture model using the Rotary Cell Culture System (RCCS) bioreactor. This custom designed RCCS anaerobic vessels were used to test the effect of aloin on the fecal microbiota. As a proof of concept, we incubated 3% rat fecal-slurry with 0.5, 1, and 2 mg/ml of aloin to test antimicrobial properties. The 16s rRNA sequencing result was consistent with *L. acidophilus* growth curve data and live bacterial counts, where aloin either lacked microbicidal property or increases the bacterial growth. Similarly, *Bifidobacterium choerinum* and *B. thermophilum* also increased due to the aloin treatment at 24 hr samples. In contrast, *Clostridium indolis, C. sulfatireducens, Bacteroides xylanolytics*, and *Alkalibacter saccharofermentans* species decreased in abundance after 24 hr. Aloin decreased butyrate-production in fecal microbiota in a dose-dependent manner after 24 hr exposure. Furthermore, *Enterococcus faecium* was capable of degrading aloin into aloe-emodin at a slower-rate compared to *Eubacterium spp.* The present study provides evidence that aloin exhibits antibacterial properties toward commensal bacteria, depending upon the growth conditions and concentration of aloin.

Email: kuppan.gokulan@fda.hhs.gov

EVALUATING THE INHIBITORY EFFECT OF THE FLOATING TETRACYCLINE DELIVERY SYSTEM ON *HELICOBACTER PYLORI* IN A SIMULATED GASTRIC ENVIRONMENT VIA 3D PRINTING MODEL

Kao, C.Y.;*[1,2] Hsu, Y.T.;[1] Chiu, T.S.;[1] Hsu, C.C.[1]
[1] Graduate Institute of Biomedical Engineering, National Taiwan University of Science and Technology, Taipei, Taiwan
[2] Department of Biomedical Engineering, National Defense Medical Center, Taipei, Taiwan

Helicobacter pylori (H. pylori) is a gram-negative microaerophile bacteria that has curved-rod shape. *H. pylori* is the only kind of bacterium discovered to survive in the human stomach, where they live in the epithelial cell of the gastric mucosa. An *H. pylori* infection may put people into the higher risk of gastritis, gastric and duodenal ulcers, gastric cancer and gastric mucosa related lymphoma among other conditions [1]. Gastric cancer is currently among the top five most common cancers in the world. At present, the most common treatments of *H. pylori* infection are the standard triple therapy with proton pump inhibitor and antibiotics; the other way is standard quadruple therapy with proton pump inhibitor, bismuth salt, and antibiotics. However, the bactericidal-success-rate of these two standard therapies isn't consistent. Furthermore, the ephemeral time of residence of the drugs in the stomach cause that is easily eliminated before reaching the concentration level that can effectively inhibit *H. pylori* [2]. Therefore, the first part of this study was designed to reduce the side effects caused by the fast absorption of the drugs *in vivo* using the prepared Tetracycline-Alginate floating beads and wrapping the drugs with vectors that control drug release as slowly release. We also carried out the assessment of the beads surface and *in vitro* tests. In the second part of this study, we performed the *in vitro* experiments of *H. pylori* by culturing *H. pylori* in a simulated 3D model of a gastric environment and then observed and recorded the *in vitro* growth of the bacteria under different conditions. As shown by our results, the surface of the Tetracycline-Alginate floating beads that floated on the medium is full of micropores; the slowly release effects were reflected by the higher accumulated release rate of the beads with the addition of HPMC. Regarding the encapsulation efficiency, the results showed that the beads with chitosan had higher drug encapsulation efficiency. According to the *in vitro* study results, *H. pylori* grew best in 5% gastric mucosa, but cannot survive in the environment of pH value of 1~2. If the quantities of mucosa added into the medium that was changed, a trace-amounts of *H. pylori* may survive even in an environment with a pH value of 2. In the future, experiments, we will further study the inhibitory effect of Tetracycline-Alginate floating beads on the growth of *H. pylori* in a simulated gastric environment. We anticipate these finding might enhance the clinical success and generate a new therapeutic strategy for treating *H. pylori* infection.

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Email: ckao@mail.ntust.edu.tw

DESIGN AND CONDUCT OF A LARGE PHASE 3 EFFICACY STUDY OF AN INVESTIGATIONAL *CLOSTRIDIUM DIFFICILE* VACCINE (CLOVER)

Kitchin, N.R.E.;*[1] Webber, C.;[1] Bailey, R.;[1] Smith, H.;[2] Rarrick, K.;[3] Lockhart, S.;[1] Gruber, W.C.;[4] Anderson, A.S.;[5] Jansen, K.U.;[5] Pride, M.W.[5]
[1]Vaccine Clinical Research & Development (R&D), Pfizer, Hurley, UK
[2]Clinical Development & Operations (CD&O) Project Management, Pfizer, Hurley, UK
[3]CD&O Study Management, Pfizer, Collegeville, PA USA
[4]Vaccine Clinical R&D, Pfizer, Pearl River, NY USA
[5]Vaccine R&D, Pfizer, Pearl River, NY USA

Clostridium difficile infection (CDI) causes diarrhea and colitis, which can be life-threatening, mostly in patients who have had recent healthcare contact and/or received antibiotics. CDI has been recognized by the Centers for Disease Control and Prevention as an urgent public health threat. Clover (B5091007, NCT03090191) is a multinational Phase 3 study evaluating the efficacy, safety and tolerability of an investigational toxoid-based *C. difficile* vaccine. Since no immunological correlate of protection for CDI exists, evaluation of vaccine efficacy requires collection and microbiological testing of diarrheal specimens.

The study population and sample size were determined based upon the anticipated number of confirmed cases of CDI and were informed by epidemiological studies and literature review. Sophisticated stool collection, transport, and testing methodologies were developed to assess all episodes when a subject experiences ≥3 unformed stools (Bristol stool chart types 5-7) in 24 hours. An electronic method to maintain contact with subjects and facilitate recording of diarrheal episodes was developed.

Eligibility for inclusion was based on healthcare contact in the previous 12 months, planned hospitalization, or receipt of systemic antibiotics in the previous 12 weeks. The target enrolment of 17,476 subjects ≥50 years of age was achieved in 24 months. A stool collection kit was developed and refined after field testing. Since *C. difficile* toxins are heat labile, a simple-to-use self-cooling shipping unit that activates upon pressing a button was developed and validated. A two-step diagnostic stool testing algorithm was developed and validated: the first detects toxigenic *C. difficile* by polymerase chain reaction and the second measures the presence of toxin A and/or B using a proprietary cell cytotoxicity neutralization assay. An app, used on a subject's own smartphone or a provided device, was developed to allow recording episodes of diarrhea, triggering reminders to collect stool samples and pick up, and to remind subjects periodically to demonstrate their continued participation in the study.

Clover will provide a robust evaluation of the efficacy of an investigational *C. difficile* vaccine to prevent CDI.

Email: nicholas.kitchin@pfizer.com

ACCURACY AND REPRODUCIBILITY OF 16S RRNA GENE PROFILING OF BACTERIAL COMMUNITIES: A LABORATORY INVESTIGATION

Liu, C.;*[1] Srinivasan, S.;[1] Fiedler, T.L.;[1] Proll, S.C.;[1] Hoffman, N.G.;[1,2] Balkus, J.E.;[1,2] Fredricks, D.N.[1,2]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]University of Washington, Seattle, WA USA

Few studies have examined the accuracy and reproducibility of bacterial community profiling using 16S rRNA gene sequencing when multiple methods are used to enumerate input bacteria. We compared three methods to quantify bacteria in mock communities. We also determined the accuracy and reproducibility of 16S rRNA gene profiling in mock bacterial communities and human vaginal swabs.

Mock communities representing *Lactobacillus*-dominant, intermediate and bacterial vaginosis-like communities were assembled with known cultivated bacteria. Each bacterium was measured using the direct cell count method (microscopy), colony forming units (CFU) in culture, and targeted quantitative PCR (qPCR). *In vivo* specimens collected from participants representing different vaginal communities were also evaluated. DNA extraction on each sample was performed on separate aliquots by two lab technicians blinded to the samples (personnel replicates), each in duplicate (technical replicates). Broad-range PCR targeted the V3-V4 region of the 16S rRNA gene. Taxonomy was assigned using a custom reference set and a phylogenetic placement tool.

Some bacteria in mock communities had higher CFU vs. the direct count (E.g. *Lactobacillus gasseri*, 8.8×10^5 vs. 1.1×10^5), while others had lower CFU (E.g. *Gardnerella vaginalis*, 5.9×10^3 vs. 9.8×10^4), particularly in low diversity communities. Differences in community representation were also noted with qPCR measurements (E.g. Relative abundance of *Prevotella bivia*: 22% by CFU vs. 20% by direct count vs. 10.4% by qPCR). All taxa in the mock communities were detected by 16S rRNA gene sequencing, although there was variability in proportions between actual counts (direct, CFU, qPCR) and sequence data. There was high reproducibility between technical and personnel replicates even at low relative abundance (<1%).

Discrepancies in CFU vs. direct count may be due to chaining of bacteria, cell viability, bacterial replication between cell counting and CFU plating or dilution errors. We recommend that each lab validates their approach for bacterial community profiling.

Email: congzhou@fredhutch.org

IN SILICO PREDICTION AND *IN VITRO* ASSESSMENT OF MICROBIAL SUBSTRATE UTILISATION: A FOCUS ON NEWLY IDENTIFIED HEALTH PROMOTING GUT BACTERIA

Lordan, C.;*[1,2] Thapa, D.;[2] Walsh, A.M.;[2] Ross, P.R.;[3] Cotter, P.D.[2,3]
[1]School of Microbiology, University College Cork, Ireland
[2]Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland
[3]APC Microbiome Ireland, Cork, Ireland

The contribution of the gut microbiota to health and disease is becoming ever more apparent in the last number of years, due to developments in DNA sequencing technology and more well-defined cultivation techniques. This has resulted in the identification of health-promoting bacteria. Until recently, prebiotics, non-digestible food substrates which are selectively utilised by beneficial bacteria, were employed with a view to increasing the growth of well-established health promoting bacteria, namely *Lactobacillus* and *Bifidobacterium*. However, other beneficial bacteria recently revealed may also be targeted to enhance their growth as they establish themselves as the next generation of health-promoting microbes. These include anaerobes such as *Akkermansia muciniphila, Faecalibacterium prausnitzii* and *Eubacterium rectale*. Identification of growth substrates/bioactives through the analysis of genome sequence data can aid in elucidating which substrates may best enhance the growth of these microbes which are often difficult to grow.

The phenotypic microbial trait analyser, Traitar, can predict 67 phenotypes based on the genome sequence inputted. Some of these traits include substrates that could potentially be utilised by the bacteria. Another tool, CarveMe, which has been created with the aim of making metabolic modelling more user-friendly, was also used with the same genomes. A select number of substrates identified in both tools have been chosen to be evaluated *in vitro* in order to establish the accuracy of these predictive tools as well as giving an indication as to how these beneficial microbes can be modulated through dietary components.

Email: cathy.lordan@teagasc.ie

THE DIFFICILE GENOMICS SEQUENCING AND TYPING SERVICE

Perry, M.D.;[1] Morris, T.;*[1] Connor, T.;[2] Bull, M.;[2] Corden, S.;[2] Hughes, H.C.[1]
[1]UK Anaerobe Reference Unit (UKARU) Public Health Wales, Cardiff, UK
[2]Pathogen Genomics Unit (PENGU), Public Health Wales, Cardiff, UK

'DIGEST' is our new real time national typing service for *Clostridioides difficile* utilising whole genome sequencing – the DIifficile GEnomics Sequencing and Typing Service.

C. difficile infection (CDI) remains a priority for healthcare institutions worldwide. The ability to distinguish between transmission, re-infection, and recurrence is key to instigating the appropriate infection prevention and control (IP&C) response where resources are limited.

PCR ribotyping developed in UKARU and refined by others is now the most commonly used method for typing *C. difficile* across Europe. However, newer methods that utilise whole genome sequencing (WGS) provide far higher discriminatory power and allow us to examine the ecology of *C. difficile* within our healthcare systems with increased certainty.

The Welsh Government and Public Health Wales have prioritised the utilisation of genomics technology to influence public health in Wales, with the development of services for several key pathogenic bacteria and viruses, including *C. difficile.*

The DIGEST service includes centralised culture and sequencing of all first line positive stool samples from symptomatic patients from across Wales within the UKARU and Pathogen Genomics Unit (PENGU). The in-house bioinformatics pipelines developed allow comparison of sequences and clustering of cases down to zero single nucleotide polymorphism (SNP) level, with results delivered in real time. IP & C teams receive relatedness information via ICNET (clinical surveillance software), whilst the wider clinical teams can visualise additional information including prescribing history and patient movements via Tableau (business intelligence and analytics software).

The DIGEST service has already proven invaluable in tracking transmission events and has revealed that the ecology and transmission of *C. difficile* is more complex than previously conceived. Health Protection teams within Wales are currently developing resources to investigate interinstitutional transmission events.

Email: trefor.morris@wales.nhs.uk

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ANAEROBIC BACTERIA—THE DEVELOPMENT OF A NEW METHOD AND REVISION OF THE EUROPEAN COMMITTEE FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING GUIDELINES

Copsey-Mawer, S.; Morris, T.;* Hughes, H.C.; Perry, M.D.; Howe, R.A.
On behalf of the EUCAST sub-committee for susceptibility testing of Anaerobic Bacteria.
UK Anaerobe Reference Unit (UKARU), Public Health Wales, Cardiff, UK

A sub-committee of EUCAST was formed in 2019 with the objectives of developing a reference MIC method and to review current EUCAST guidelines including Epidemiological Cut Offs (ECOFFS), breakpoints and methodology.

During 2019 interested parties were invited to become members of the sub-group and terms of reference were discussed and agreed. The sub-committee currently includes members from Sweden, Hungary, Turkey, Denmark, France, the Netherlands, and Wales.

Current agar dilution methodology utilises supplemented Brucella blood Agar (BRU), developed in house by Sydney Finegold, which supports the growth of most anaerobic organisms. Fastidious Anaerobe Agar (FAA), developed in the UK, was specifically designed for the cultivation of anaerobic bacteria from primary samples, but was also examined initially for its suitability for AST.

EUCAST principles for AST include recommending a media that performs well across a wide range of species whilst also being commercially available from more than one manufacturer. FAA fulfils these criteria with the added advantage of not requiring the addition of key supplements (as per BRU) such as haemin and vitamin K or specific species dependant supplements such as cysteine (*Fusobacterium species*) and pyruvate (*Bilophila species)*. FAA will be examined further in 2020 via inter-laboratory comparisons and, if successful, adopted for both the reference testing method and disk susceptibility testing.

The sub-group has also collated and interrogated MIC data shared by its members and is using this data to review current EUCAST ECOFFs and Breakpoints for anaerobes. Alongside determining whether the data is still valid we will be making recommendations for anaerobic bacteria that require individual breakpoints.

Email: trefor.morris@wales.nhs.uk

COMPARATIVE EVALUATION OF SEMI-AUTOMATED AND AUTOMATED SYSTEMS FOR IDENTIFICATION OF CLINICALLY RELEVANT GRAM POSITIVE ANAEROBIC COCCI

Akgul, O.; Ulger Toprak, N.*
Marmara University School of Medicine Department of Microbiology, Istanbul, Turkey

Susceptibility profiles of Gram positive anaerobic cocci (GPAC) may vary at the species level, therefore rapid and accurate identification of GPAC plays a critical role in successful treatment. Here, we describe the identification ability of MALDI TOF MS (VITEK® MS) system, API® RAPID ID 32 A and the BBL Crystal™ Anaerobe for GPAK using the 16S rRNA gene sequencing as a reference method.

A total of 153 non-duplicate clinically relevant GPAC were tested. The isolates were representatives of 5 different genera. In total 66 (43.1%) strains' 16S rRNA gene sequence showed 99% similarity with *Parvimonas micra,* and 49 isolates (32.2%) were identified as *Finegoldia magna* with 99% smilarity. However, the sequence of 23 isolates (15%) showed 99% identity to sequences of *Peptoniphilus* harei. and *Peptoniphilus asaccharolyticus*. Similarly, the sequence of 14 (9.1%) isolates were found to be 99% similar to that's of *Peptostreptococcus anaerobius* and *Peptostreptococcus stomatis*. An isolate showed 91% similarity to a sequence of *Anaerococcus octavius.* The VITEK MS results agreed with 16S rRNA sequencing for *P. micra and F. magna*. The remaining isolates were identified as *P. asaccharolyticus* (15%) and *P. anaerobius* (9.1%), respectively, with the exception of an isolate identified as anaerococcus by sequencing.

The isolates were identified to the genus level for *Parvimonas, Finegoldia, Peptoniphilus, Peptostreptococcus* as 40.1%, 19.7%, 5.7%, 11.2% and 41.4%, 26.3%, 7.9%, 2.6% by RapID and Crystal, respectively.

Identification performances of semi-automated systems were found high for *P. micra*. However, their identification power may vary according to the species. Since genus-level information is usually sufficient for clinical diagnostic purposes, semi-automated systems are more suitable for small laboratories. Although 16S rRNA gene sequencing is highly useful in regards to bacterial identification, it has poor discriminatory power for *P. harei* and *P. asaccharolyticus*, as well as *P. anaerobius* and *P. stomatis.* The MALDI-TOF MS system has performed well in identification of GPAC, as a reliable alternative to the 16S rRNA gene sequencing.

Email: nurverulgerahoo.com

Anaerobic Pathology

PROBING THE *STREPTOCOCCUS ANGINOSUS* GENOME TO UNDERSTAND ITS INVASIVE POTENTIAL

Abdelrahman, Y.M.;*[1,3] Li, J.;[1] Brenner, A.E.;[2] Raghavan, R.;[2] Merritt, J.L.[1]
[1]Department of Restorative Dentistry, Oregon Health and Science University, Portland, OR USA
[2]Department of Biology, Portland State University, Portland, OR USA
[3]Department of Microbiology and Immunology, Faculty of Pharmacy Cairo University, Cairo Egypt

Streptococcus anginosus is a typical member of the human oral microbiome and one of the most frequent sources of life-threatening streptococcal invasive disease, largely as a consequence of its unusually high prevalence and abundance in both odontogenic and extraoral abscesses. Despite this, surprisingly little is understood about the invasive potential of these organisms. Using comparative genomics of sequenced strains of *S. anginosus*, our aim was to identify putative *S. anginosus* loci that are either positively or negatively associated with invasive disease.

10 clinical isolates of *S. anginosus* (5 invasive and 5 abscess) were sequenced using Illumina HiSeq, annotated using the NCBI Annotation Pipeline, and then analyzed together with other publicly available *S. anginosus* genome data to compare the core and non-core genomes of noninvasive and invasive strains. As part of this study, we also developed a new strategy to greatly simplify gap closure between contigs during genome assembly.

403 mobile genetic elements (MGE) were identified and classified among the 40 published *S. anginosus* genomes. Of these, 255 are tyrosine integrases (T-MGEs), 82 are serine recombinases (S-MGEs), and 31are DDE transposase-based MGEs in addition to 24 phages. The boundaries of all identified T-MGEs were mapped and found to target >15 unique genes, whereas S-MGEs targeted an additional >10 genes. Interestingly, multiple S-MGEs were found to frequently integrate into the loci of other S-MGEs. At least 5 novel, previously undescribed MGE target insertion sites were identified. Five *S. anginosus* strains harbored ≥2 phages per genome, while 12 strains harbored a single phage. Genome-wide comparative analyses of the non-core genes identified several genes uniquely associated with the invasive isolates, as well as additional loci uniquely associated with noninvasive isolates. These findings are currently being independently verified, using a cohort of 35 invasive *S. anginosus* clinical isolates derived from bacteremic blood specimens collected in a hospital clinical pathology laboratory over a two-year period.

Email: abdelray@ohsu.edu

DETECTION BY *IN VITRO* AMPLIFICATION OF THE ALPHA TOXIN (PHOSPHOLIPASE C) GENE AND ENTEROTOXIN GENE OF *CLOSTRIDIUM PERFRINGENS* TYPE A FROM POULTRY DROPPINGS IN SOUTHWEST NIGERIA

Egwuatu, T.O.G.;[1] Amodu, D.Z.;[1] Effiniyini, E.;[1] Olaleye, O.A.;[1] Olorunsogo, A.O.;[1] Orkeh, G.O.;[2] Egwuatu, C.A.;[3] Ogunsola, F.T.[4]
[1]Department of Microbiology, Faculty of Science, University of Lagos, Nigeria
[2]Department of Healthcare Administration, California State University, Long Beach, CA USA
[3]Lifegate Specialist Hospital, Ikeja, Lagos, Nigeria
[4]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria

Clostridium species produce toxins that are responsible for diseases in poultry, as well as abdominal cramping and diarrhoea in humans. Though diarrhoeal diseases induced by Clostridia are not uncommon worldwide, they are hardly reported in Nigeria. This study characterized *Clostridium perfringens* from poultry droppings. A total of 105 chicken and turkey droppings were collected from 3 different poultry farms, out of which 65 were analyzed for the presence of *Clostridium* species using 16S rRNA and triosephosphate isomerase (*tpi*) housekeeping genes. Multiplex polymerase chain reaction assay was also performed to detect the phospholipase C (*plc*) gene and enterotoxin gene. The result revealed 86% similarity to *Clostridium perfringens*, 100% similarity to *Clostridium beijerinckii*, 100% similarity to *Clostridium butyricum*, 100% similarity to *Clostridium drakei*, and 96% similarity to *Clostridium sphenoides*. There was a positive correlation between the 16sr RNA gene and the tpi gene, statistical significance ($p=0.001$, $\alpha=0.01$). The plc gene revealed 10 (15.4%) *C. perfringens* were Type A strains, while the *cpe* gene revealed 6 (9.2%) were enterotoxigenic *C. perfringens* Type A, with broiler chickens having the highest prevalence of 4 (16.7%). There was also a positive correlation between the *plc* and *cpe* gene, statistical significance ($p=0.02$, $\alpha=0.05$). Alpha toxin is considered the most virulence factor in the pathogenesis of necrotic enteritis (NE) in poultry, while enterotoxin is responsible for food poisoning in humans. The detection of the enterotoxin gene of *C. perfringens* type A is of public health importance because faecal material containing the cpe gene can contaminate meat obtained from broiler chicken during slaughtering, thereby resulting in food illnesses such as diarrhoea and abdominal cramps in humans.

Email: tegwuatu@unilag.edu.ng

GENOMICS OF UNCULTIVATED *GRACILIBACTERIA* SHOWS DIVERSITY AND BROAD HUMAN PRESENCE

Hendrickson, E.L.;* Kerns, K.; To, T.; McLean, J.S.
Department of Periodontology, University of Washington, Seattle, WA USA

We assembled genomes of phylum *Gracilibacteria*, a member of the large, but poorly understood, bacterial Candidate Phylum Radiation (CPR), from human oral anaerobic biofilms to gain a better understanding of the CPR, *Gracilibacteria*, and the oral and environmental differences in the phylum. Genomes were assembled from metagenomic samples using MegaBLAST to find *Gracilibacteria* hits to 16S rRNA genes in contigs. Raw reads were then re-assembled with SPAdes, binned into genomes, manually curated, and a phylogeny constructed based on concatenated single copy ribosomal genes. The results showed that *Gracilibacteria* had small genomes, consistent with other CPR genomes and the epibiont lifestyle of the first cultured CPR member. Previous 16S studies had revealed multiple class level divisions within *Gracilibacteria*, two of which contained both environmental and oral species. Phylogeny showed the genomes split between these two groups, G1 and G2. This was supported by GC content differences, G1 at 21-25% and G2 at 36-38%, as well as comparing orthologous genes. The phylogenetic tree confirmed the G1/G2 split but also showed sub-class level divisions between oral and environmental species. Mapping sequences from human body sites to the genomes showed broad presence across the human oral cavity, as well as presence in other body sites. The oral G1 had their highest coverage in the tongue dorsum, anterior nares, and mid vagina, the oral G2 in the posterior fornix, and tongue dorsum. This study provides new insight into this mostly unknown phylum showing that *Gracilibacteria* from the oral cavity are distinct from environmental species, even within the same group, and are present across multiple human body sites, including non-oral sites.

Email: elh@uw.edu

THE *CLOSTRIDIUM PERFRINGENS* TWO COMPONENT REGULATORY SYSTEM VIRS MEMBRANE PROTEIN IS A SIGNALING PEPTIDE RECEPTOR FOR THE AGR-LIKE QUORUM SENSING SYSTEM

Li, J.;* Mehdizadeh Gohari, I.; McClane, B.A.
Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA USA

Previous studies showed that the Agr-like Quorum Sensing (QS) system of *Clostridium perfringens* controls production of many toxins, including beta toxin (CPB). The Agr-like QS becomes activated when 5R, a 5-mer cyclic signaling peptide (SP), binds to an unidentified receptor. Since production of several *C. perfringens* toxins, including CPB, is positively regulated by both Agr-like QS and VirR/S two component regulatory system, the VirS membrane protein has been suggested as a/the SP receptor. To begin evaluating whether VirS is a/the SP receptor for the *C. perfringens* Agr-like QS system, we first sequenced the *virS* genes in CN3685 and CN1795 to seek an explanation for why *agrBD* mutants of both *C. perfringens* strains respond to 5R, the natural 5-mer SP, but only the CN3685 *agrBD* mutant can respond to 8R, which is SP plus a 3 amino acid tail. This sequencing identified a 6 amino acid insert that is present in the predicted second extracellular loop (ECL2) of VirS of CN3685, but not CN1795. Based upon the differences in SP-based peptide sensitivity and ECL2 sequences between the two *C. perfringens* strains, we constructed *virS* and *agrB* double null mutants of each strain and then complemented those strains to swap which VirS protein they express. Western blotting for beta toxin production was showed to change the natural responsiveness of the strains to the 5R SP or 8R SP. We used a Biotin-5R peptide in pull down experiments to show VirS directly binds to SP, which further confirmed VirS as an Agr-like QS SP receptor. To further demonstrated this relationship, and identify the VirS binding site for SP, we synthesized a 14-mer peptide corresponding to ECL2. This 14-mer peptide blocked the ability of 5R to signal an *agr* mutant of these strains to produce CPB and this peptide also blocked CPB production by wild-type strains. This inhibition was specific since a single N to D substitution caused loss of function for this 14-mer peptide. Collectively, these results support VirS as an important SP receptor, especially for toxin production.

Email: jihongli@pitt.ed

EFFECTS OF DIFFERENT SIZES OF NANO ZINC OXIDE ON THE BIOFILM FORMATION BY *STREPTOCOCCUS MUTANS* IN SALIVA ANALOG MEDIUM

Park, M.;* Rafii, F.; Paredes, A.; Sutherland, J.B.
National Center for Toxicological Research, Jefferson, AR USA

Purpose of the study. This study was conducted to evaluate the effects of different sizes of nanoparticle zinc oxide (nZnO), currently used in dentistry, on the growth and biofilm formation by *Streptocococcus mutans* ATCC 25175.

Methods and Results. *S. mutans* was incubated anaerobically in BHI medium overnight at 37°C for inoculum. Basal medium mucin (BMM), with and without 1% sucrose as a carbon source in 48-well, microtiter plates were inoculated with 10^5-10^6 CFU/mL of the bacterium. nZnOs, with sizes of 70 nm, 40-100 nm, and 20-30 nm, were purchased from Alfa Aesar as a form of colloidal (70 nm) or powder (40-100 nm and 20-30 nm). Powder forms of nZnO were dispersed in water and sonicated for 15 min at 48W and 35kHz before use. The 24 h growth dynamics were determined spectrophotometrically at an optical density reading of 600 nm. The metabolic activity of sucrose fermentation was measured by end point pH. The phenol-sulfuric acid method was used to quantitate extracellular polysaccharide in biofilm. A colorimetric assay using **1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT)** was employed to measure the viability of *S. mutans* in the biofilm. Gene expression involved in biofilm formation was measured by RT-qPCR. The inhibitory effects of different sizes of nZnOs on bacterial growth were different, with the colloidal 70 nm ZnO being the highest. The end point pHs of *S. mutans* in BMM were 4.2 and 4.0, with or without 32 mg/mL nZnO, respectively. The number of live bacteria from the planktonic and biofilm were not significantly affected by nZnO. MTT staining results were consistent with the bacterial counting results. RT-qPCR results indicate a up to 10-fold decrease in expression of *gtfBCD* and *ftf* (genes involve with biofilm formation) when *S. mutans* was grown in nZnO during the exponential phase of growth but expression recovered to levels comparable to growth in the absence of nZnO by the end of the exponential phase of growth.

Conclusion. Results of the study indicate that nZnO had antibacterial activity against *S. mutans*. The inhibitory activity increased as the concentration of nanoparticles was increased.

Email: miseon.park@fdahhs.gov

RECIPROCAL POSTTRANSLATIONAL CONTROL OF *STREPTOCOCCUS MUTANS* LRS AND NATURAL COMPETENCE

Qin, H.;* Merritt, J.L.
Department of Restorative Dentistry, Oregon Health & Science University, Portland, OR USA

Objectives: The development of natural competence provides bacteria with a mechanism to repair damaged genes or acquire new advantageous traits, such as antibiotic resistance. In the human cariogenic species *Streptococcus mutans*, natural competence development is critically dependent upon the natural competence-specific sigma factor ComX. BrsRM, a member of the recently described LytTR Regulatory Systems (LRS), was previously investigated for its role in bacteriocin production and the activation of cell death in *S. mutans*. Recently, we also observed potent stimulation of *S. mutans* natural competence via the BrsRM LRS. Our objective was to determine the mechanism of this ability.

Methods: Mass spectrometry analysis of BrsR coimmunoprecipitates (Co-IP) was employed to identify candidate protein-protein interaction partners. Candidate interactions were further verified using specific Co-IP assays. Genetic regulatory mechanisms were characterized using a combination of luciferase assays, western blots, and electrophoretic mobility shift assays (EMSA).

Results: Mass spectrometry analyses indicated that BrsR directly interacts with a variety of late competence proteins as well as ComX. A subset of 6 natural competence-related proteins were chosen from the list of potential interactors and confirmed via direct Co-IP studies with BrsR. Due to protein-protein interactions, BrsR increases the abundance of the ComX protein by inhibiting ComX degradation. This stimulates late competence protein production and the induction of natural competence. Upon their production, late competence proteins like DprA directly bind to BrsR to antagonize its autoregulatory activity.

Conclusions: BrsR activates the *S. mutans* natural competence system through its ability to directly stabilize ComX. The late competence proteins produced, as a result of ComX stabilization, can then inhibit BrsR autoregulation as a mechanism to reset the circuit. This posttranslational regulatory mechanism is a new paradigm for *S. mutans* natural competence development, which could conceivably be employed by other protein regulators of natural competence that act via unknown mechanisms.

Email: qinh@ohsu.edu

MICROBIOME OF TRANSGENIC NON-STEROIDAL ANTI-INFLAMMATORY DRUG ACTIVATED GENE 1 MICE

Reese, S.D.;* Snodgrass, C.; Binder, A.K.; Pinkart, H.C.
Department of Biological Sciences, Central Washington University, Ellensburg, WA USA

Recent research has shown that the composition of the gut microbiome influences obesity. A transgenic mouse model with the human non-steroidal anti-inflammatory drug (NSAID) activated gene 1 (known as NAG-1) has demonstrated an increased metabolism and obesity resistance when compared to the original strain of mice, C57BL/6, termed wild type (WT). In this study, we examined the microbiome of NAG-1 mice to better understand their resistance to diet-induced obesity and increased metabolism. Both male and female NAG-1 mice were compared to their WT litter mates to analyze metabolism, body weight, glucose tolerance, and their microbiomes. A total of 31 mice (6-8 mice per group) were used in the experiment. Mice were housed in the Central Washington University (CWU) vivarium under conditions approved by the Institutional Animal Care and Use Committee. Body weight and glucose tolerance were measured at weekly intervals throughout the experiment. Mice were euthanized at the end of the study, and cecum and colon were aseptically removed for gut microbiome analysis. 16S ribosomal RNA (rRNA) gene amplicons were sequenced to compare the gut microbial communities of each group. At the conclusion of the experiment, there was significant difference in weight between the sexes. Increased glucose tolerance was observed in the male NAG-1 mice versus the WT males. With regards to the microbiome, initial results indicate a trend toward greater alpha diversity in NAG-1 mice compared to the WT mice. A significant difference in beta diversity between groups was observed as well. The *Bacteriodetes:Firmicutes* ratio was observed to be higher in the NAG-1 and WT males in comparison to the female groups. Additionally, the NAG-1 males had a higher ratio of *Bacteriodetes:Firmicutes* than the WT males. This observed difference in microbial communities likely contributes to the observed differences in glucose tolerance and weight observed in males.

Email: safyre.reese@cwu.edu

FIVE-YEAR RETROSPECTIVE SURVEY OF ANAEROBIC BACTERIA ISOLATED FROM BLOOD CULTURES IN A UNIVERSITY HOSPITAL

Sayin, E.;[1] Bilgin, H.;[2] Altinkanat-Gelmez, G.;[1] Ulger Toprak, N.;*[1,3] Soyletir, G.[1]
[1]Marmara University Pendik Education and Research Hospital, Istanbul, Turkey
[2]Marmara University, School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey
[3]Marmara University, School of Medicine, Department of Microbiology, Istanbul, Turkey

The aim of this study is to determine the prevalence of bacteremia due to anaerobic bacteria and evaluate the clinical characteristics of these patients in a 650-bed tertiary care university hospital in Istanbul, Turkey.

We examined the obligate anaerobic bacteria isolated from routine anaerobic blood culture from July 2014 to November 2019, retrospectively. Adult inpatients who developed anaerobic bacteremia with accessible clinical information from patient charts were included to the study. Mortality in anaerobic bacteremia patients compared with Mann-Whitney U and T test for continuous variables and chi-square test and Fisher-exact test for categorical variables.

During 5-year period a total of 37,376 anaerobe blood cultures submitted to the laboratory. From those cultures 302 (0.81%) obligate anaerobic isolates were detected. *Cutibacterium* spp. accounted for 35.4 % of isolates (reported mainly as contaminants), followed by *Bacteroides* spp. (24.5%), *Parvimonas micra* (6.6%), *Clostridium* spp. (8.9%), *Prevotella* spp. (6.3%), *Fusobacterium* spp. (4.6%), *Eggerthella lenta* (3.3%), *Peptoniphilus* spp. (2.7%) and *Veillonella* spp. (2.3%). Other Gram positive and Gram negative anaerobes were in a rate of 4% and 1.3%, respectively. In 8 patients, multiple bacteremia caused by mixed anaerobic organisms observed. Univariate analysis shows that median age, Pitt score and presence of gastrointestinal (GIS) malignancy was associated with the 30-day mortality rate. In multivariate analysis, Pitt bacteremia score and GIS malignancy remained significant risk factors for 30-day mortality.

Routine anaerobic blood cultures may be most necessary for patients with complex underlying disease and who often are immunocompromised, especially having GIS malignancy. Furthermore isolation of anaerobic organisms will lead to the administration of the appropriate antibiotic.

Email: nurverulgerahoo.com

OVEREXPRESSION OF LRS REGULATOR BRSR ACTIVATES CELL DEATH IN *STREPTOCOCCUS MUTANS*

Xu, F.;*[1,2] Zou, Z.;[1] Merritt, J.L.[1]
[1]Department of Restorative Dentistry, Oregon Health and Science University, Portland, OR USA
[2]School of Animal Science and Technology, Anhui Agricultural University, Hefei, Anhui, China

Background: Many bacterial species undergo cell death as part of a defined, regulated pathway. Recently, we described a new signal transduction system, LytTR Regulatory System (LRS), represented by BrsRM and HdrRM in *Streptococcus mutans*. BrsR positively regulates natural competence and bacteriocin production. Intriguingly, overexpression of BrsR also induces a potent suicide-like cell death pathway.

Methods: We constructed a xylose-inducible *brsR-hdrR* system, which can mimic BrsR overexpression-induced cell death. Using this induction system, we performed a genome-wide transposon mutagenesis and screened for mutations that could survival under high xylose concentration.

Results: We created a mariner transposon library of >20,000 mutants and identified 52 death-resistant mutants. Of these, Clp protease encoding genes *clpX* and *clpP* were the most frequently found, indicating their importance in the BrsR-induced cell death pathway. Other mutants included *liaF*, encoding a membrane protein of the LiaFSR three-component signaling system; *comX*, encoding an alternative sigma factor; SMU_399, encoding a putative C3-degrading proteinase; and the promoter region of *dnaK*, which encodes a chaperone protein. The role of these genes suggested that BrsR-induced cell death may be linked to bacterial competence. We, therefore, tested the core competence genes and found that a DcomS mutant was also resistant to cell death but not *comCDE* or *comR* mutants. Further investigation of ComX showed that the internal peptide XrpA encoded within the *comX* gene is responsible for cell death resistance, but not ComX itself. We also tested the contribution of *recA* and *mazEF*, which are key regulators of previously described bacteria cell death pathways, and found they were not involved in BrsR-induced cell death.

Conclusion: The results of this study provide insights into the BrsR-induced cell death pathway. While details of the regulatory mechanism are currently under investigation, our data showed that BrsR-mediated cell death is distinct from previously described bacteria cell death pathways as key regulators of these pathways were not shown to influence BrsR-induced cell death. Collectively, our results suggest that cell death of *S. mutans* induced by BrsR overexpression may represent a new pathway of programmed cell death.

Email: xufa@ohsu.edu

Clinical Abstracts

Clinical Abstracts

PANDRUG RESISTANT *BACTEROIDES FRAGILIS* ISOLATES ON THE RISE IN THE NETHERLANDS

Boiten, K.E.;*[1] Kuijper, E.J.;[2,3] Smit, L.F.E.;[3] Bode, L.G.M.;[4] Schuele, L.;[1] Schoffelen, A.F.;[2] Notermans, D.;[2] Woudt, S.H.S.;[2] Winter, H.L.J.;[1] van Prehn, J.;[3] Veloo, A.C.M.[1]

[1]University of Groningen, University Medical Center Groningen, Department of Medical Microbiology and Infection Prevention, Groningen, the Netherlands

[2]Centre for Infectious Disease Control (CIb), National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

[3]Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands

[4]Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands

Three different pandrug resistant *Bacteroides fragilis* isolates with a rare resistance pattern, including resistance to metronidazole and carbapenem, were isolated in three Dutch hospitals. Whole genome sequencing will be used to assess the mechanisms of resistance.

Isolate A (2019) was cultured at the LUMC from a patient recovering from a liver transplant. Isolate B (2018) was found at the Erasmus MC from a patient with malignancies in the urogenital region and isolate C (2014) was isolated from a patient with a complicated appendicitis at the UMCG. A hybrid assembly of short and long reads will be used to obtain a closed genome and assess the presence of extra-chromosomal elements in all 3 isolates.

The patients from whom isolate A and B were cultured died due to underlying illness. The patient from whom isolate C was cultured, recovered without specific antimicrobial treatment. All three isolates were resistant or intermediate to amoxicillin, amoxicillin-clavulanic acid, meropenem, clindamycin, metronidazole, tetracycline and moxifloxacin. Using short read sequencing we found that all isolates harboured the following AMR genes: *cfiA* gene with IS element, *ermA*, *nim* (isolate A and C *nimE*, isolate B *nimA*), *tetQ* and *bexA*. Isolate A and C also harboured a *mef* gene, respectively *mefE* and *mefA*, isolate B and C harboured *cfxA* and isolate B *ermF*.

The rise of pandrug resistant anaerobes is worrying, definitely with regard to clinically relevant species such as *B. fragilis*. It is important to better understand the origin and dissemination of resistance linked to these strains. The hybrid assembly results will be presented.

Email: k.e.boiten@umcg.nl

THE SUBTYPE DISTRIBUTION OF *CUTIBACTERIUM ACNES* ON THE SKIN IS ASSOCIATED WITH THE RISK OF PROSTHETIC FAILURE

Bumgarner, R.E.;*[1,2] Anderson, K.A.;[1] Harrison, D.;[1] Matsen III, F.A.;[2] Hsu, J.E.[2]
[1]Department of Microbiology, University of Washington, Seattle WA USA
[2]Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, WA USA

Cutibacterium acnes is frequently cultured from failed shoulder and spine implants, and previous research has suggested that some subtypes are enriched in implant failure (relative to the distribution on the skin). Previous research on implant infections has looked at the distribution of subtypes of individual deep isolates from collections of implant revision patients and has compared this to the subtype distribution on the skin of unrelated subjects. Most humans harbor more than one subtype on and in the skin. In previous research on implant infections, it has been unclear if the enrichment of certain subtypes at deep sites was due selection as the subtypes moved from the skin to the deep environment OR if the presence of certain subtypes on the skin increased the risk of subsequent implant infection.

To address this issue, we measured the subtype distribution on the skin of 58 patients undergoing shoulder revision surgery and 46 patients undergoing primary shoulder surgery. In brief, skin swabs were collected prior to surgery and prior to skin disinfection. DNA was isolated from the swabs and PCR was used to amplify a highly polymorphic region of the *C. acnes* genome that has been previously used for Single Locus SubTyping (SLST). The PCR products were sequenced on an Illumina miSeq sequencer and the sequence data from 100's to 1000's of reads per subject were analyzed to determine the subtype distribution on the skin. Unsupervised clustering of the subtype distribution data yielded two main subpopulations, one of which was dramatically enriched in patients undergoing surgical revision ($P<0.001$) . These results strongly suggest that subtype distribution on the skin is a risk factor for subsequent infection.

Email: rogerb@uw.edu

THE ROLE OF ANAEROBES IN THE PATHOGENESIS OF CHRONIC RHINOSINUSITIS (CRS)

Cho, D.Y.;*[1,2] Skinner, D.;[1] Weeks, C.;[1] Swords, W.E.;[2,4] Hunter, R.C.;[3] Rowe, S.M.;[2,5] Woodworth, B.A.[1,2]
[1]Department of Otolaryngology, University of Alabama at Birmingham (UAB), Birmingham, AL USA
[2]Gregory Fleming James CF Research Center, UAB, Birmingham, AL USA
[3]Department of Microbiology & Immunology, University of Minnesota, Minneapolis, MN USA
[4]Departments of Medicine & Microbiology, UAB, Birmingham, AL USA
[5]Departments of Medicine, Pediatrics, Cell Developmental & Integrative Biology, UAB, Birmingham, AL USA

Background: Chronic rhinosinusitis (CRS) is characterized by complex bacterial communities that incite persistent inflammation and airway damage. Mucin-degrading anaerobes (MDA) are predominant during the early phase of acute rhinosinusitis in a rabbit model and may provide carbon source nutrients (e.g. short-chain fatty acids, SCFAs) to bacterial pathogens observed after 12 weeks of chronic infection. The objective of this study is to evaluate the capability of MDA to contribute to the growth of *Pseudomonas aeruginosa*.

Methods: Rabbit rhinosinusitis was induced by blocking the sinus cavity for 2 weeks to create an anaerobic environment for MDA. Control and sinusitis mucus were collected and co-cultured with PAO1 strain of *P. aeruginosa* for 72 hours and colony forming units were determined. Targeted quantification of SCFAs in control and sinusitis mucus was performed via high performance liquid chromatography.

Results: Colony counts per tube were significantly higher in those tubes contained with the mucus samples from week 2 (8.4x109+/-4.8x107) compared to tubes containing control mucus (1.4x109+/-2.0x107) or no mucus (1.5x109+/-2.1x107) ($p<0.0001$). Acetate concentrations were significantly greater in the rabbit mucus samples collected on week 2, relative to day 0 (4.13+/-0.53 vs 1.94+/-0.44 mM, $p<0.01$). All SCFAs were significantly higher in CRS compared to controls in human (acetate, $p<0.05$; propionate, $p<0.0001$; butyrate, $p<0.01$).

Conclusion: Given that SCFAs are exclusively derived from bacterial fermentation, our evidence suggests a critical role for mucin-fermenting bacteria in generating carbon-source nutrients for pathogenic bacteria. MDA may contribute to the development of CRS by degrading mucins, thus providing nutrients for potential pathogens like *P. aeruginosa*.

Email: dycho@uabmc.edu

REMOVAL CHARACTERISTICS AND MECHANISM OF HEXAVALENT CHROMIUM BY ANAEROBIC *OCHROBACTRUM* SP. YC213

Chen, C.Y.;[1] Chiu, C.H.;[2] Cheng, C.Y.;[2] Chen, P.Y.;[2] Kuo, J.T.;[2] Chung, Y.C.*[2]
[1]Department of Tourism and Leisure, Hsing Wu University, New Taipei, Taiwan
[2]Department of Biological Science and Technology, China University of Science and Technology, Taipei, Taiwan

Hexavalent chromium [Cr(VI)] is extensively used in diverse industries, and it is a toxic, mutagenic and carcinogenic environmental pollutant. *Ochrobactrum* sp. YC213 was isolated from the vicinity of an electroplating factory. In this study, the removal characteristics and mechanism of Cr(VI) by *Ochrobactrum* sp. YC213 were determined under anaerobic conditions to evaluate its feasibility. To clearly illustrate the feasibility of strain YC213 to remove high Cr(VI) concentration from wastewater, the removal efficiency, distribution and fate of Cr(VI) reduced by YC213 were analyzed and determined by SEM, EDS, TEM, SAED, FTIR, XRD and UV-vis spectroscopy. Additionally, Cr(VI) reductase and related gene in YC213 was identified. The results indicated that a high removal efficiency (99.1±0.3%) corresponding to 147.6±12.6 mg-Cr/g-dry cell weight-h was achieved by YC213 at 1200 mg/L Cr(VI) under anaerobic condition. Almost Cr(VI) was reduced and precipitated as $Cr(OH)_{3(s)}$ form accounting for 84.71% of total Cr, and most precipitates were located inside cells (71.58%). 8.13% of total Cr were adsorbed by the cell surface as free-ion Cr^{6+} form, and 11.38% were adsorbed by the cell surface in exchangeable, adsorbed, organically-bonded, or carbonate Cr(III) forms. 4.87% of total Cr was soluble Cr(III) complex existed as $Cr(OH)_4^-$, $Cr_2O_2(OH)_4^{2-}$ or $Cr_3O_4(OH)_4^{3-}$. The results also indicated *Ochrobactrum* sp. YC213 utilized ChrR and CrS reductases to efficiently removal Cr(VI) under anaerobic condition. By genetic analysis, the main local genomic context of the *chrR* genes contained a group of the *chrA*, *chrB*, and *chrC* genes. The *chrR* gene from *Ochrobactrum* sp. YC213 had 98.4%, 96.8%, and 90.7% similarity with that from *E. coli*, *Pseudomonas* sp., and *Gluconacetobacter* sp. by BLASTN analysis, respectively.

Email: ycchung@cc.cust.edu.tw

DETECTION OF BACTERIAL SPECIES IN THE GUT MICROBIOME PRECEDING BACTEREMIA IN ADULT TRANSPLANT RECIPIENTS

DeMeules, M.M.;*[1] Pergam, S.A.;[1,2] Quinn, Z.Z.;[1] Proll, S.C.;[1,2] Loeffelholz, T.;[1] Srinivasan, S.;[1] Liu, C.;[1] Fiedler, T.L.;[1] Hoffman, N.G.;[1,2] Fredricks, D.N.[1,2]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]University of Washington, Seattle, WA USA

Bacteremia is common among hematopoietic cell transplant (HCT) recipients, despite frequent use of empiric antibiotics. Characterizing gut bacteria prior to bacteremia may provide an avenue for identifying likely agents of infection, thereby helping to guide optimal initial antibiotic therapy.

Patients undergoing allogeneic HCT were enrolled in a longitudinal study of the gut microbiota, with weekly sampling of stool. We reviewed medical records from 287 adults in this study to identify patients who experienced at least one episode of bacteremia. Stool samples underwent DNA extraction and 16S rRNA gene PCR with sequencing to identify bacterial species present in the gut. We excluded patients who developed coagulase-negative *Staphylococcus* bacteremia and those who did not have at least one stool sample within 30 days prior to bacteremia.

Our final cohort was comprised of 48 patients with 60 bacteremia episodes. In 20 of 60 episodes, the organism associated with bacteremia was one of the top five abundant species in the gut in that sample. The most common bloodstream infections were caused by *Enterococcus faecium* (n=6), *Escherichia coli* (n=5), and *Klebsiella pneumoniae* (n=8). In 15 of 19 bacteremia episodes caused by these species, the isolated organism was detected in the stool sample preceding bacteremia by no more than 20 days. Intestinal domination of at least 30% relative abundance was present in 9 of 13 bacteremia episodes, involving *E. coli*, *E. faecalis*, and *E. faecium*, and the implicated organism was the most abundant species in 8 of these episodes.

The gut serves as a potential reservoir for microbes that cause bloodstream infections, particularly in HCT recipients whose gut microbiota is disturbed post-transplant. Detecting the presence and abundance of key bacterial species in the gut may help providers predict bacteremia in this population.

Email: mdemeule@fhcrc.org

CLINICAL ANALYSES OF A DEFINED BACTERIAL CONSORTIUM IN HEALTHY VOLUNTEERS

Dsouza, M.;*[1] Bhattarai, S.K.;[2] Menon, R.;[1] Schneider, J.;[1,3] Minot, S.S.;[4] Olle, B.;[1] Bobilev, D.;[1] Bucci, V.;[2] Roberts, B.;[1] Norman, J.M.[1]
[1]Vedanta Biosciences, Inc. Cambridge, MA USA
[2]University of Massachusetts Medical School, Department of Microbiology and Physiological Systems, Worcester, MA USA
[3]Current: Gastroenterology Drug Discovery Unit, Takeda Pharmaceuticals International Co., Cambridge, MA USA
[4]Microbiome Research Initiative, Fred Hutchinson Cancer Research Center, Seattle, WA USA

Live biotherapeutic products (LBPs) consisting of defined consortia of clonal bacterial isolates have been proposed as a novel class of therapeutics for the treatment of diseases such as *Clostridioides difficile* infection. Here, we propose a framework and set of statistical analyses based for the evaluation of strain detection and colonization, host-microbiota interactions, and microbiome recovery associated with LBPs in humans. We apply these methods to clinical data from a Phase 1 study with VE303 - a defined LBP comprising 8 characterized, non-genetically engineered, clonally-derived, nonpathogenic, non-toxigenic, commensal strains of Clostridia currently in clinical development for the prevention of recurrent *C. difficile* infection. We show that the strains colonize rapidly and increase in abundance following LBP administration in healthy volunteers pretreated with vancomycin. We also show durability of colonization extending out to at least 1 year after LBP administration. VE303 administration accelerates the pharmacodynamic recovery of the vancomycin-perturbed resident microbial community and is associated with the enhanced recovery of secondary bile acids in the gut compartment and the reduction of proteobacterial antibiotic resistance genes.

Email: mdsouza@vedantabio.com

THE *VEILLONELLACEAE:* AN EMERGING PATHOGENIC GENUS WITH CHANGING SUSCEPTIBILITY PATTERNS

Goldstein, E.J.C.;*[1,2] Citron, D.M.;[1] Merriam, C.V.[1]
[1]RM Alden Research Laboratory, Santa Monica, CA USA
[2]UCLA School of Medicine, Los Angeles, CA USA

An increasing number of *Veillonella* species (12 to 14) are being recovered, most of which cannot be identified via conventional biochemical and phenotypic methods, including an increasing number from human infections. These lactate utilizing (conversion to propionate and acetate by the methylmalonyl-CoA pathway) Gram-negative cocci may be normal components of the oral and intestinal flora in mammals, but also have been etiological agents in endocarditis, discitis, osteomyelitis, sepsis and from tongue biofilms associated with halitosis. Of 305 *Veillonella* isolates in our collection, 118 had "no good match" and 141 were identified by PCR gene-sequencing. Our lab identified 5 different species, plus 11 "no- species match" isolates from clinical specimens obtained from multiple body sites during the years 1984 to 2017, and tested them against 9 antimicrobial agents. *V. parvula* was the species most readily identified using Rapid ANA kits, and the most frequently identified species (93/141, 65.9%). It was recovered from abdominal infections (22), respiratory (15) blood (11) human bites (12) and multiple tongue cultures (as part of a halitosis study). Other commonly isolated *Veillonella* sp. were *V. atypica* (19), *V. dispar* (13), *V. rogosae* (4) and *V. tobetsuensis* (1). Breakpoints for piperacillin-tazobactam (P-T) CLSI (>64 μg/ml) and EUCAST (>16 μg/ml) differ making the interpretation of the literature problematic. Overall resistance was exhibited against P-T (10% CLSI vs. 44% EUCAST) and penicillin-G (46% both). There was considerable resistance to moxifloxacin (27% CLSI, no EUCAST breakpoint) and doxycycline (23% CLSI, no EUCAST breakpoint). Moxifloxacin resistance of *V. parvula* rose from 15% (1984-20 00) to 39% (2001-2017) in *V. dispar (11% to 50%)* and *V. atypica* (29% to 42%). Resistance was more likely in abdominal isolates, (P-T, 13% CLSI vs 50% EUCAST; moxifloxacin, 30% CLSI ; and doxycycline (23% CLSI), respiratory (P-T, 10% CLSI vs 50% EUCAST; moxifloxacin, 30% CLSI; doxy, 20% CLSI), and blood cultures (P-T 12% CLSI vs 29% EUCAST; moxifloxacin, 24% CLSI; and doxycycline 12% CLSI) tended to be more resistant than those recovered from other sources. No meropenem and rare metronidazole (1 isolate) resistance was encountered. *V. parvula* exhibited sporadic resistance to ampicillin-sulbactam and linezolid.

Email: ejcgmd@aol.com

THE IMPACT OF NANOSCALE TIO_2 AND ZNO USED IN SUNSCREENS ON SKIN BACTERIA

Jin, J.;*[1] Li, L.;[1] Hu, H.;[2] Cerniglia, C.E.;[1] Chen, H.[1]
[1]Division of Microbiology, National Center for Toxicological Research, US FDA, Jefferson, AR, USA
[2]Regulatory Implementation Staff, Office of Dietary Supplement Programs, Center for Food Safety and Applied Nutrition, US FDA, College Park, MD USA

Nanoscale titanium dioxide (TiO_2) and zinc oxide (ZnO) are two major physical ultraviolet (UV) filters used in sunscreens. Despite the beneficial health effects of these nanoparticles, little is known about their impacts on the skin microbiome when used topically. Reported antimicrobial activities of nanomaterials have raised safety concerns on the potential disturbance on aerobic and anaerobic skin bacteria, which play important roles in skin and systemic human health. This study aimed to evaluate the impact of nanoscale TiO_2 and ZnO on the viability of skin bacteria and assess the effects of UV light on the antimicrobial activities of nanoparticles.

Brain heart infusion (BHI) agar plates covered with different concentration of nanoparticles were used to mimic sunscreen application on human skin. The antimicrobial activities of 10 coated or uncoated nanoparticles were evaluated by performing plate counting assay with BHI-nanoparticle agar plates against 10 common skin bacterial strains under anaerobic or aerobic conditions. The results showed that uncoated ZnO particles showed dose-dependent antimicrobial activities. One hour-exposure to UVA (320-400 nm) enhanced bactericidal activities of uncoated ZnO (3.1 mg/mL) against *Cutibacterium acnes*, *Staphylococcus haemolyticus*, *S. warneri*, and *S. epidermidis* by 2-5 log numbers. Without UV exposure, uncoated TiO_2 particles (12.5 mg/mL) decreased 2-3 log numbers of *Micrococcus lylae* and *Kocuria kistinae*. 10 minutes-exposure to UVB (311 nm) enhanced bactericidal activities of uncoated ZnO (3.1 mg/mL) against *C. acnes* by 2 log numbers. Two coated TiO_2 particles jeopardized the UVB bactericidal effects by increasing 1- 2 log numbers of bacteria cells. These results suggested that nanoparticles in sunscreen products combining the influences of UV radiation may alter skin microbiome in a complex manner. The information from this study could advance our knowledge for the assessment of nanoparticles interaction with skin microbiome.

Email: jinshan.jin@fda.hhs.gov

THE EFFECT OF INTERVENTION BY AN ANTIMICROBIAL STEWARDSHIP TEAM ON ANAEROBIC BACTEREMIA

Kuwahara, G.;* Shibata, W.; Yamada, K.; Kakeya, H.
Institution; Infection Control Science, Osaka City University Graduate School of Medicine, Japan

The effect of antimicrobial stewardship (AS) on anaerobic bacteremia is uncertain. This study aimed to assess the effect of interventions by the AS team (AST) on clinical and microbiological outcomes and antimicrobial use. An AS program was introduced at Osaka City University Hospital in January 2014; an interdisciplinary AST was established. We enrolled patients with anaerobic bacteremia between January 2009 and December 2018. Patients were classified into the pre-intervention group (from January 2009 to December 2013) and the post-intervention group (from January 2014 to December 2018). A significant decrease in definitive carbapenem use (P=0.0242) and an increase in empiric tazobactam/piperacillin u (P=0.0262) were observed in the post-intervention group. The de-escalation rate increased significantly from 9.38 % to 32.7 % (P=0.0316) in the post-intervention group. The susceptibility of Bacteroides species and 30-day mortality did not worsen in the post-intervention group. These results showed that interventions by an AST can reduce carbapenem use and increase the de-escalation rate without worsening patient outcomes.

Email: g.kuwabara1226@gmail.com

URETHRAL MICROBIOTA IN NONGONOCOCCAL URETHRITIS (NGU) IN MEN WHO HAVE SEX WITH MEN (MSM) AND MEN WHO HAVE SEX WITH WOMEN (MSW)

Srinivasan, S.;[1] Chambers, L.C.;[2] Tapia, K.;[2] Hoffman, N.G.;[2] Munch, M.M.;*[1] Morgan, J.L.;[3] Domogala, D.;[1] Lowens, M.S.;[3] Proll, S.C.;[2] Huang, M.L.;[2] Soge, O.O.;[3] Jerome, K.R.;[2] Golden, M.R.;[2] Hughes, J.P.;[2] Fredricks, D.N.;[1,2] Manhart, L.E.[1]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]University of Washington, Seattle, WA USA
[3]Public Health Seattle & King County Health Dept., Seattle, WA USA

NGU is common with no known etiology in ~50% of cases. We evaluated the association of urethral bacteria with NGU among MSM and MSW.

Urine samples were collected from men attending a Seattle STD Clinic and enrolled in a case-control study. *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) were detected by TMA (Aptima); *Trichomonas vaginalis* (TV), adenovirus and HSV by PCR. NGU was defined as reported urethral symptoms or visible discharge and ≥5 PMNs/high powered field (HPF). Absence of CT, MG, TV, adenovirus, and HSV in the setting of NGU was considered idiopathic NGU. Men without NGU had no urethral symptoms or discharge and <5 PMNs/HPF. Broad-range 16S rRNA gene PCR with deep sequencing was used to characterize the urethral microbiota. Compositional lasso analysis of bacterial taxa was conducted to identify associations between bacteria and NGU; beta coefficients (β) giving change in probability of NGU/log2 change in relative abundance are reported. Bacterial taxa positively associated with NGU were measured by quantitative PCR (qPCR).

Of 434 men (199 MSM, 235 MSW) with urine samples, 250 men had NGU while 113 had idiopathic NGU. Higher relative abundances of *Haemophilus influenzae* (HI) (β=0.01575) and *Mycoplasma penetrans* (MP) (β=0.01575) were positively associated with NGU in MSM with no detection of CT or MG (non-CT/MG), while HI was positively associated with non-CT/MG NGU in MSW (β=0.01767). Notably, *Lactobacillus iners* was inversely associated with non-CT/MG NGU in MSW (β=-0.00202), but not MSM. No other bacterial community types or specific anaerobes were associated with NGU. HI and MP were selected for measurements using qPCR. Quantities of HI (p=0.005) and MP (p=0.034) were associated with idiopathic NGU. MP detection was associated with idiopathic NGU (p<0.001) only in MSM.

HI and MP are potential causes of male urethritis. MP may be more common in MSM than MSW.

Email: mmunch@fredhutch.org

CYCLED TOBRAMYCIN PRIMARILY AFFECTS UNTARGETED, ANAEROBIC BACTERIA IN THE CYSTIC FIBROSIS SPUTUM MICROBIOME

Nelson, M.T.;* Eng, A.; Ratjen, A.; Ravishankar, S.; McNamara, S.; Nay, L.; Majors, C.; Vo, A.; Hayden, H.S.; Brittnacher, M.J.; Weiss, E.; LiPuma, J.J.; Simon, R.H.; Borenstein, E.; Hoffman, L.
University of Washington, Seattle, WA USA
University of Michigan, Ann Arbor, MI USA

Cystic fibrosis respiratory microbial communities are known to be relatively stable, even during antibiotic therapy, but less is known about the metagenome (the microbial community's predicted functional capacities). Inhaled tobramycin is known to reduce sputum densities of the canonical aerobic pathogen *Pseudomonas aeruginosa* and to improve CF lung disease measures on average in treatment-naïve patients, yet studies have not demonstrated a consistent relationship between culture-based microbiological changes and clinical outcomes. We hypothesized that metagenomic analysis would better characterize microbial community responses to inhaled tobramycin, which are not well characterized by culture. We collected sputum samples prior to, during, and following a standard 1-month course of inhaled tobramycin from 30 people with CF, comprising 157 samples. We characterized the microbiota and metagenome using culture, quantitative PCR, and shotgun metagenomic sequencing. Viable counts decreased after one week of therapy, with a plateau or return to baseline levels after treatment. We did not observe a reduction on average in total bacterial load by qPCR. We identified a substantial shift in microbiota constituency by one week on therapy that did not recover substantially by the end of therapy and that was driven primarily by changes in relative abundance of non-dominant, anaerobic taxa, with notable interpatient heterogeneity. Functional metagenomic characterization also revealed high interpatient heterogeneity in community functional capacity, but little change in overall metagenomes by week on therapy, suggesting functional redundancy within the CF sputum microbiome. Metagenomes did change significantly with treatment only after excluding reads from dominant taxa, supporting the conclusion that tobramycin primarily affected anaerobic bacteria without substantially changing overall predicted community functional capacity. Standard clinical culture methods did not effectively reveal these changes. Our results suggest that tobramycin maintenance treatment may not stabilize lung disease by changing abundances of dominant pathogens, such as *P. aeruginosa*.

Email: mnelson8@uw.edu

CHARACTERISATION OF *CLOSTRIDIUM* ISOLATES FROM A SURVEY AT A PUBLIC HEALTH CARE FACILITY IN CAPE TOWN, SOUTH AFRICA.

Monki, T.;[1] Beylis, N.;[1,2] Moodley, C.;[1,2] Paul, L.V.*[1,2]
[1]Division of Medical Microbiology, University of Cape Town, South Africa
[2]Microbiology Laboratory (National Health Laboratory Services, Groote Schuur Hospital), Capetown, South Africa

Published data on anaerobe clinical isolates from South Africa is limited. A previous survey, done in the setting of Groote Schuur Hospital, indicated that *Bacteroides fragilis* and clostridia (e.g. *Clostridium perfringens*) were the most prevalent anaerobe bacteria isolated from suspected anaerobe infections in this setting. Anaerobes from that survey exhibited 100% sensitivity against the beta lactam-inhibitor combinations and less than 5% resistance to metronidazole. Here, we report sensitivity data of 20 clostridia isolates from a survey of anaerobic bacteria, done during the period January 2018 – December 2019.

Methods: Anaerobic bacteria were obtained from samples submitted for routine diagnostic testing to the Microbiology Laboratory of the NHLS, situated at Groote Schuur Hospital (Cape Town, South Africa). The identity of isolates was confirmed using ANC ID cards and the VITEK2 instrument (Biomerieux). Antimicrobial sensitivity testing (AST) was done using MIC test strips (Liofilchem, Italy) and Brucella agar with sheep blood. Results were interpreted according to Clinical & Laboratory Standards Institute (CLSI).

Results: Data for 20 clostridia tested against amoxicillin-clavulanate, piperacillin-tazobactam, penicillin, clindamycin, metronidazole and imipenem are reported here. The *C. baratii* isolate exhibited sensitivity to all antimicrobials tested, while *C. sporogenes* were sensitive to all drugs, except clindamycin. Of the *C. perfringens* isolates, 10 were sensitive to all antimicrobials, 2 were resistant to metronidazole only (>256μg/ml), 2 were multidrug-resistant (metronidazole, clindamycin and penicillin) and 4 were resistant to clindamycin.

Conclusion: Metronidazole resistance of *C. perfringens* in our clinical setting have increased. Continued surveillance is needed to monitor drug resistance of clostridia in our clinical setting and generate a larger dataset to evaluate AST data. This will aid the implementation of appropriate measures to curb increased metronidazole resistance.

Email: lynn.paul@uct.ac.za

NEXT GENERATION SEQUENCING ANALYSIS OF A *CLOSTRIDIUM BOTULINUM* OUTBREAK ASSOCIATED WITH HOME CANNED PEAS

Perry, M.J.;* Centurioni, D.A.; Conlon, M.A.; Rourke, A.K.;
LaPierre, P.; Egan, C.T.
New York State Department of Health—Wadsworth Center, Albany, NY USA

In 2018, just 14 hours after consuming home canned peas, several patients presented to the hospital with symptoms characteristic of botulism. It was suspected that these individuals had consumed food containing botulinum neurotoxins (BoNT), which disrupted communication at neuromuscular junctions resulting in descending paralysis.

Blood and stool specimens were collected and shipped to the New York City Department of Health and Mental Hygiene and the New York State Department of Health (NYSDOH). Real-time PCR (rtPCR) analysis was performed at the NYSDOH to screen for the presence of botulinum neurotoxin (*bont*) genes. In all specimens, *bont* A and B genes were detected. BoNT activity was confirmed using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF/MS); however, it was determined that only active BoNT/A was present in the patient specimens. After epidemiological investigation and environmental sampling, testing was performed on food and tableware collected from the home. The isolates recovered from stool specimens and environmental samples were tested and agreed with all other testing results.

To epidemiologically link and characterize BoNT-producing isolates, a whole genome sequencing pipeline was developed. Enrichment broths were inoculated with isolated colonies and grown at 35°C under anaerobic conditions for 24 hours. Bacterial cells were pelleted and manually extracted prior to preparing libraries using Nextera XT preparation kits. All samples were sequenced using an Illumina MiSeq instrument using 500 cycle v2 reagent kits.

Toxin serotypes for each isolate were assigned by comparing kmers generated from raw reads to unique kmers identified in each *bont* gene. SNP-based analysis was used to compare isolates obtained from outbreak samples. No SNP differences were detected, suggesting that the isolates were indistinguishable. In combination with epidemiological data, we suggest that this analysis provides sufficient evidence to definitively link patient and environmental isolates.

Email: michael.perry@health.ny.gov

Clostridioides difficile Management

DIFFERENT PROBIOTIC STRAINS ALTER THE RETURN OF COLONIZATION RESISTANCE AGAINST *CLOSTRIDIOIDES DIFFICILE* AFTER ANTIBIOTICS

Foley, M.H.;*[1] O'Flaherty, S.J.;[2] Allen, G.;[1] Barrangou, R.;[2] Theriot, C.M.[1]
[1]College of Veterinary Medicine, Department of Population Health and Pathobiology, NCSU, Raleigh, NC USA
[2]Department of Food, Bioprocessing, & Nutrition Sciences, NCSU, Raleigh, NC USA

Clostridioides difficile is a spore-forming, toxin-producing bacterial pathogen that is a major public health threat. *C. difficile* often initiates infection after antibiotics perturb the gut microbiota and metabolome, resulting in the loss of colonization resistance. Probiotics are widely used after antibiotics to reconstitute the gut microbiota and prevent antibiotic-associated diarrhea. However, the effect of probiotic treatment on the post-antibiotic gut microbiota is poorly understood, and recent studies have suggested that probiotic administration may delay its reconstitution. **Therefore, the purpose of this study is to determine if and how popular probiotic Lactobacilli strains are able to accelerate or prolong the return of colonization resistance against *C. difficile* after antibiotic treatment**. Cefoperazone-treated mice were challenged with probiotic strain *Lactobacillus acidophilus* NCFM or *Lactobacillus gasseri* NCK2638. Following a one-time probiotic administration, groups of mice were then challenged with *C. difficile* each consecutive week for one month. Mice were monitored for clinical signs of disease, fecal bacterial burden, and toxin activity to measure CDI. Using 16S rRNA sequencing and targeted bile acid metabolomics, we defined how different *Lactobacillus* strains were able to alter the return of the gut microbiota and bile acid pool after antibiotics. Our results show that *L. acidophilus* delayed the return of colonization resistance against *C. difficile*, while *L. gasseri* accelerated the return, which was associated with the return of bile acids (CDCA, DCA and LCA) that are inhibitory to *C. difficile*. We are continuing to investigate the bile acid-altering enzymes encoded by these Lactobacilli in order to define the mechanisms that underlie the return of colonization resistance against *C. difficile*. Future work will include engineering enhanced probiotics able to accelerate the return of colonization resistance after antibiotic treatment.

Email: mfoley3@ncsu.edu

MILD OR MALIGN: CLINICAL OUTCOMES OF *CLOSTRIDIUM DIFFICILE* INFECTION IN THAILAND

Imwattana, K.;*[1,2] Putsathit, P.;[3] Leepattarakit, T.;[2] Kiratisin, P.;[2] Riley, T.V.[1,3,4,5]
[1] University of Western Australia, Crawley, WA, Australia
[2] Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[3] Edith Cowan University, Joondalup, WA, Australia
[4] Murdoch University, Murdoch, WA, Australia
[5] PathWest Laboratory Medicine, Nedlands, WA, Australia

Little is known about *Clostridium difficile* and its infection (CDI) in Asia, and specifically Thailand. Several molecular epidemiological studies have suggested that the prevalence of *C. difficile* strains in Asia differs from those in the other continents, and anecdotal clinical evidence indicates that disease is milder. This case-control study was conducted to evaluate the clinical outcomes of CDI in Thailand. A total of 469 patients with diarrhea were included in this study. CDI cases were defined as patients whose stool were positive for toxigenic *C. difficile* by both direct *tcdB* PCR and toxigenic culture. All patients were assessed for severity of disease using Zar criteria, treatment administered and clinical outcomes. A total of 248 patients were confirmed to have CDI, leaving 221 patients in the control group. The CDI group had a higher 30-day all-cause mortality rate than the control group (21% vs 14%, $p = 0.046$). However, only two deaths (1%) were directly attributed to CDI and the most common cause of death in both groups was septic shock due to other infections. Patients with severe CDI had a higher 30-day mortality rate than patients with non-severe CDI (36% vs 8%, $p < 0.001$). Oral metronidazole was chosen as an initial treatment in 184 (74%) patients, 28 (15%) of whom did not respond and oral vancomycin was later given. A total of 60 (24%) patients received oral vancomycin. Patients treated with metronidazole did not have poorer outcomes in term of mortality or recurrence rate compared to patients treated with vancomycin. In conclusion, CDI in Thailand was a relatively mild disease. The Zar criteria were an effective tool for predicting clinical outcomes in CDI cases. Metronidazole remained an effective treatment choice in both severe and non-severe CDI with a low failure rate. Further studies should be done to identify factors that might be protective against CDI in this population.

E-mail: korakrit.imwattana@research.uwa.edu.au

PATIENTS WITH SEVERE *CLOSTRIDIOIDES DIFFICILE* INFECTION HAVE INCREASED RISK OF SYSTEMIC INFECTIONS WITH MULITDRUG-RESISTANT ORGANISMS

Kuo, J.;* Gonzales-Luna, A.J.; Dotson, K.; McPherson, J.; Begum, K.; Alam, M.J.; Garey, K.W.
University of Houston College of Pharmacy, Houston, TX USA

Objective: The aim of this study was to evaluate colonization rates of multi-drug-resistant organism (MDRO) stool colonization in patients with *Clostridioides difficile* infection (CDI) and incidence of MDRO systemic infection in the 30-days after CDI diagnosis.

Methods: This was a prospective, observational study of adult hospitalized patients tested for CDI at a large, university-affiliated tertiary care hospital in the Texas Medical Center, Houston Texas. Patients with positive CDI toxin tests were identified and followed for 30 days after the positive test. Stool samples were collected and cultured for *C. difficile* and selected MDROs (Enterococcus, Candida, and Staphylococcus aureus). Polymerase chain reaction (PCR) and specific primers were used to detect resistant genes in stool samples. Patients with a history of systemic MDRO infection in the past 7-90 days of stool testing were excluded.

Results: A total of 335 CDI-positive hospitalized patients were analyzed (Healthcare facility onset: 26.0%, Community onset: 59.1%, Community onset unknown: 14.6%). The most prevalent MDROs were VRE, Candida, and MRSA. For non-severe CDI, there were 32.1% VRE, 19.0% Candida, and 12.7% MRSA species respectively. For severe CDI, there were 23.0% VRE, 18.9% Candida, and 14.9% MRSA species respectively. For fulminant CDI, there were 28.9% VRE, 10.5% Candida, and 18.4% MRSA species respectively. The incidence of systemic MDRO infection was 7.2% in non-severe CDI and 18.6% in severe and fulminant CDI.

Conclusions: Severe CDI disease is associated with an increased risk of systemic MDRO infections. There was a consistent percentage of VRE, Candida and CRE organisms in stool colonization for patients with non-severe, severe and fulminant CDI. Most CDI episodes were in community onset patients.

Email: jkkuo@central.uh.edu

IMPLEMENTATION OF MODIFIED ALGORITHM TO IMPROVE DIAGNOSIS OF CLOSTRIDIUM DIFFICILE INFECTION AT HAHNEMANN UNIVERSITY HOSPITAL

D'Mello, K.;[1] Shah, S.N.;[1] Goldstein, N.D.;[2] Ahmed, M.;[1] Weldie, C.;[1] Kim, J.;[1] Quinn, A.;[1] Adeli, B.;[1] Rossi, J.;[1] Lin, B.;[1] Krevolin, K.;[3] Jones, H.;[4] Pride, M.W.;[4] Mohamed, N.;[4] Anderson, A.S.;[4] Nandi, N.;[1] Welles, S.L.;[2] Kutzler, M.A.*[1]
[1]Drexel University College of Medicine, Philadelphia, PA USA
[2]Drexel University Dornslife School of Public Health, Philadelphia, PA USA
[3]Hahnemann University Hospital, Philadelphia, PA USA
[4]Vaccine Research and Development, Pfizer, Inc., Pearl River, New York USA

Clostridioides difficile infection (CDI) rates have increased over the last 2 decades due to the emergence of hypervirulent strains, increased antibiotic use, and increased nosocomial exposure. At Hahnemann University Hospital, it was noted that the volume of *C. difficile* diagnostic tests was disproportionally high for subjects taking laxatives and that the incidence of repeat testing performed in confirmed CDI cases was also high. In May 2016, a new algorithm was introduced: testing was discontinued on stool samples from patients receiving laxatives and on samples obtained within 5 days of a prior test. A quality improvement study was conducted to detect the number of CDI diagnoses 6 months prior to and following the May 2016 algorithm introduction. A database was compiled for analysis and included CDIFF QUIK CHEK COMPLETE, Cepheid PCR test, and clinical data, such as laxative use. A total of 1768 patients were reviewed, 919 (52%) in PRE and 848 (48%) in POST. 236 tests were positive (155 PRE, 81 POST ($p<0.05$)). There were 498 canceled tests (214 PRE, 284 POST ($p<0.05$)). Laxative use in patients was seen in 627 cases (366 PRE, 261 POST ($p<0.05$). Although the intervention was not associated with a lower number of *C. difficile* tests being ordered in patients not on laxatives, it was effective at reducing the number of tests being processed due to a greater number of canceled tests which no longer met the testing criteria. Morevover, intervention implementaton was not associated with a delay in diagnosis and demonstrated that ordering CDI tests should be based on CDI clinical patient risk criteria and take into consideration additional diarrhea promoting factors such as stool softeners or laxatives.

Email: mak98@drexel.edu

STRAINS OF *CLOSTRIDIUM DIFFICILE* FOUND IN PAEDIATRIC PATIENTS IN WESTERN AUSTRALIA

Perumalsamy, S.;*[1] Riley, T.V.[2,3,4]
[1]The University of Western Australia, Crawley, WA, Australia
[2]Murdoch University, Murdoch, WA, Australia
[3]Edith Cowan University, Joondalup, WA, Australia
[4]PathWest Laboratory Medicine, Nedlands, WA, Australia

Clostridium difficile infection (CDI) in children is poorly understood. A rising incidence of both hospital and community-acquired CDI in children has been reported in many countries, often in children without traditional risk factors. However, research in Australia regarding this situation is limited. Here, we report the molecular characterisation of *C. difficile* isolated from paediatric patients in Perth, Western Australia. A total of 231 stool samples was collected from paediatric patients being investigated for diarrhoea with ages ranging from <1 to 17 years from July 2019 to January 2020. All samples were enriched in a *C. difficile* selective medium (Robertson's cooked meat broth plus antimicrobials) for a week, followed by alcohol shock and culture on *C. difficile* ChromID agar. PCR toxin profiling and ribotyping was performed on isolates. Ribotypes (RTs) were then identified by comparing banding patterns to a reference library. *C. difficile* was isolated from 38 out of 231 samples (16.5%), with 47.4% of isolates (*n*=18) toxigenic (A+B+CDT-, *n*=17; A+B+CDT+, *n*=1) and 52.6% (*n*=20) non-toxigenic. Overall, 16 different RTs were identified; the most common non-toxigenic RT found was RT 010 (*n*=7, 18.4% of isolates), while the most common toxigenic RT was RT 014/020 (*n*=7, 18.4%). Interestingly, *C. difficile* RT 078, a strain that is not endemic in Australia, was isolated from a 9-month-old infant. In addition, *C. difficile* RT 106, a strain of emerging importance, in Australia was recovered from 2 cases (5.3%). The presence of well-established (in human and non-human animals) toxigenic strains like RT 014/20 suggests the possibility of paediatric patients acquiring infections from sources/reservoirs external to the hospital, contributing to the rise in CDI in Australia in recent years. Children may also play a significant role in the transmission of *C. difficile* within the community. Additional whole-genome sequencing studies are required to fully understand the epidemiology of CDI in children.

E-mail: sicilia.perumalsamy@research.uwa.edu.au

CLOSTRIDIUM DIFFICILE INFECTION: EPIDEMIOLOGY, DIVERSITY AND EVOLUTION IN ASIA

Riley, T.V.*
School of Medical and Health Sciences, Edith Cowan University, Joondalup, Australia
Department of Microbiology, PathWest Laboratory Medicine (WA), Nedlands, Australia
School of Veterinary and Life Sciences, Murdoch University, Murdoch, Australia

Clostridium difficile is the most common cause of healthcare-related diarrhoea in high-income countries. *C. difficile* infection (CDI) has been studied in detail in North America and Europe, where large outbreaks have occurred since the early 2000s, and Australia. However, the epidemiology of CDI in Asia is largely unknown, and there is little information about strains of *C. difficile* circulating in the region. In a recent survey of CDI performed in 13 countries in the Asia-Pacific region, the most common *C. difficile* strain isolated was ribotype (RT) 017 (16.7%) followed by RTs 014/020 (11.1%), 018 (9.9%), 002 (9.2%), 012 (4.8%) and 369 (4.1%), with wide variation between countries. Binary toxin-positive strains of *C. difficile* were detected rarely. Overall disease severity appeared milder, and mortality and recurrence were lower than in North America or Europe. We have studied CDI and *C. difficile* carriage extensively in Indonesia, Malaysia and Thailand. The most common strains isolated were non-toxigenic strains belonging to RTs not previously described, and there was great diversity. Again, *C. difficile* RT 017 was found often. There are at least five different clades of *C. difficile* circulating around the World. It is likely that the predominant molecular types of *C. difficile* in Asia differ from other regions of the World. The diversity of RTs found suggests that Asia is home to at least one clade of *C. difficile*, most likely clade 4, with the possibility of another clade existing in the Indian sub-continent. Some Asian strains like RT 017 have travelled to other parts of the World where they have caused major outbreaks that pre-date the spread of RT 027. This movement likely coincides with population movements, either human or animal. Continued education about, and surveillance of, CDI in Asia is required to monitor the burden of disease and prevent the emergence of virulent antimicrobial-resistant strains. As with all *C. difficile* disease, a "One Health" approach will be required to deal with this problem in Asia.

Email: thomas.riley@uwa.edu.au

CLOSTRIDIOIDES DIFFICILE COLLECTED FROM PATIENTS, THE ENVIRONMENT, AND DOGS REVEALS SHARED GENOTYPES AND PROVIDES INSIGHTS INTO TRANSMISSION POTENTIAL

Stone, N.E.;*[1] Nunnally, A.E.;[1] Hornstra, H.M.;[1] Sahl, J.W.;[1] Vazquez, A.J.;[1] Terriquez, J.;[2] Plude, C.;[2] Busch, J.D.;[1] Keim, P.;[1] Wagner, D.M.[1]
[1]Northern Arizona University, Flagstaff, AZ USA
[2]Northern Arizona Healthcare, Flagstaff, AZ USA

We systematically collected environmental samples (n=263) and dog feces (n=549) throughout Flagstaff, Arizona. These samples were processed by enriching for *C. difficile* under anaerobic conditions, extracting DNA, utilizing a TaqMan PCR to identify *C. difficile* DNA and the *tcdB* gene of the PaLoc, and isolating *C. difficile* from PCR-positive samples. This workflow yielded 185 unique isolates from these sources; we also obtained 307 isolates from toxigenic *C. difficile* positive patient stools from a local hospital. Whole-genome sequences were generated for all 492 isolates and used to assess genetic diversity among them, predict toxin status, and explore the potential for transmission among these sources, based on genomic similarities. An LS-BSR analysis was used to predict the toxin status of each isolate based on the percent identity to five toxin loci (*cdtA, cdtB, tcdA, tcdB*, and *tcdC*); a cut-off of ≥80% identity was used to determine presence. The multi-locus sequence type (ST) of each isolate was determined using *in silico* methods. 51% of environmental samples were positive for *C. difficile* with 64.2% of those being toxigenic, whereas 11.7% of dog feces were positive for *C. difficile* with 50% of those being toxigenic. We identified 49 STs from patients, 16 STs from the environment, and 13 STs from dogs. Five toxigenic (2, 8, 42, 110, and 236) and three non-toxigenic (15, 3, and 26) STs were shared among these three sources, but hypervirulent STs (from MLST clade 2) were only found in patient samples, suggesting these hypervirulent STs may not circulate commonly outside of the local clinical setting. SNP analysis within the shared STs revealed striking similarities among isolates from all three sources (<10 SNPs in some cases), suggesting either a shared source of contamination or recent transmission between them. These results suggest that non-hypervirulent *C. difficile* STs may be better adapted to survival and transmission in the environment than hypervirulent STs.

Email: nathan.stone@nau.edu

EXTENDED-PULSED FIDAXOMICIN REGIMENS TO IMPROVE CLINICAL OUTCOMES IN PATIENTS WITH MULTIPLE *CLOSTRIDIOIDES DIFFICILE* INFECTION RECURRENCES

Tan, X.;*[1] Skinner, A.;[2,3] Sirbu, B.D.;[3] Danziger, L.D.;[1] Gerding, D.N.;[2] Johnson, S.[2,3]
[1]University of Illinois at Chicago, Chicago, IL USA
[2]Edward Hines, Jr. VA Hospital, Hines, IL USA
[3]Loyola University, Maywood, IL USA

Purpose: We have previously described novel fidaxomicin regimens for managing multiply recurrent CDI patients in a preliminary small study and have now extended our experience. The dosing scheme and the time course in the disease in which the extended-pulsed (E-P) fidaxomicin regimen is administered (following CDI treatment with symptom resolution) differs from the EXTEND trial.

Methods: This is a retrospective case series of consecutive patients who received fidaxomicin in an E-P dosing scheme between January 1, 2014 through June 30, 2019 in a specialty CDI clinic at a single academic medical center. The primary outcomes include sustained clinical cure, recurrence, or failure. The episode in which the E-P fidaxomicin regimen was initially used was evaluated for the primary outcomes.

Results: Forty-six patients were included for analysis. The mean±SD age of patients was 63.67±20.01 years, 68% were female, and the mean±SD CDI episodes prior to receipt of the E-P fidaxomicin regimen was 3.82±3.12. Most (65%) of these patients had failed a prior vancomycin taper and pulse regimen. Prior to the E-P regimen, patients were given a treatment course until symptoms resolved (typically vancomycin 125 mg four times daily followed by a taper to 125 mg once daily; 27/46 patients). The median±SD CDI treatment duration was 20.00±29.44 days. The E-P regimen most often used (44/46 patients) consisted of fidaxomicin 200 mg once daily for 7 days followed by 200 mg every other day for 13 doses; the median±SD duration of this regimen was 33.00 ± 28.12 days. Of the 46 patients evaluated, sustained clinical cure occurred in 54% (25/46), recurrence in 37% (17/46), and failure in 7% (3/46). Of the patients that had a recurrence, recurrence was precipitated by subsequent antibiotic administration in 30% (5/17). The median±SD time to recurrence was 30.00±169.9 days.

Conclusions: An extended-pulsed fidaxomicin regimen may be effective in patients with multiply recurrent CDI who are refractory to other treatments, including a vancomycin tapered and pulsed strategy.

Email: xt9@uic.edu

CEFEPIME EXPOSURE IS A SPECIFIC RISK FACTOR FOR *CLOSTRIDIODES DIFFICILE* INFECTION DUE TO THE EMERGING *C. DIFFICILE* STRAIN, DH/106

Wieczorkiewicz, J.T.;*[1,2] Skinner, A.;[1,3] Cheknis, A.;[1] Stevens, V.W.;[4,5] Osmolski, J.;[3] Gerding, D.N.;[1] Johnson, S.[1,3]
[1]Hines VA Hospital, Hines, IL USA
[2]Midwestern University, Downers Grove, IL USA
[3]Loyola University Medical Center, Maywood, IL USA
[4]VA Salt Lake City Health Care System, Salt Lake City, UT USA
[5]University of Utah, Salt Lake City, UT USA

Specific antibiotics have facilitated outbreaks due to epidemic *C. difficile* strains highly resistant to those antibiotics in the past; clindamycin and the REA group J strain (RT001) in the 1990s and fluoroquinolones and the REA group BI strain (RT027) in the 2000s. We previously reported that fluoroquinolone exposure predicted infection with the BI strain in our VA hospital in 2005-7 when that strain predominated (72% of cases). We now report a follow up survey at the same hospital in 2013-15.

REA strain typing and antibiotic susceptibility testing were performed on the recovered *C. difficile* isolates from 223 first episode CDI patients at the Hines VA Hospital between 2013-15. Demographics, risk factors, and antimicrobial exposures within 6 weeks were recorded.

Three REA group strains, BI, Y, and DH accounted for 42(19%), 35(16%), and 27(12%) cases, respectively. Fluoroquinolone use was documented in 19(45%) BI-cases compared to 45(25%) non-BI cases (OR = 2.5, 95% CI 1.25 – 5). Resistance to the fluoroquinolone, moxifloxacin was found in 32(78.1%) REA BI isolates and 12(6.7%) non-BI isolates ($p < 0.001$). Third and 4th generation cephalosporin use was documented in 14(52%) DH- cases and in 47(23%) non-DH Cases (OR = 3.41, 95% CI 1.5 – 7.78). Cefepime use was documented in 7(26%) DH- cases and in 12(6.1%) non-DH Cases (OR = 5.37, 95% CI 1.9 – 15.19). Ceftriaxone exposure was similar for DH- cases (26%) and non-DH cases (18.4%; OR = 1.79, 95% CI 0.7 – 4.6). Resistance to ceftriaxone was found in 178(80.9%) of all isolates, with no significant difference between strain groups. High-level resistance to cefepime was found in a representative sample of isolates (30/30, 100%), again without differences between strains.

Although the prevalence of the epidemic BI strain (RT027) decreased markedly in our hospital, it remains associated with fluoroquinolone use. The REA group DH strain (RT106) has now emerged as a prevalent strain and is associated with cefepime use. Understanding the mechanism whereby cefepime predisposes to infection with DH is needed, but these findings highlight the interplay of antibiotic exposures and shifting epidemiology of *C. difficile* strains.

Email: jwiecz@midwestern.edu

Clostridioides difficile Pathology

Clostridioides difficile Pathology

INVESTIGATION OF *C. DIFFICILE* INFECTION (CDI) AND VANCOMYCIN-RESISTANT ENTEROCOCCUS (VRE) COLONIZATION USING QPCR FROM PATIENT STOOL DNA SAMPLES IN HOUSTON, TEXAS

Alam, M.J.;* Begum, K.; Alnezary, F.; Garey, K.W.
University of Houston College of Pharmacy, Houston, TX USA

Clostridioides difficile infection (CDI) is the most common healthcare-acquired infection in the US. Pathogenesis of CDI involves disruption of the host microbiota allowing for the growth of *C. difficile* and other multidrug-resistant organisms (MDROs). As culture and identification of *C. difficile* is difficult and time-consuming, qPCR from stool DNA may be a useful option to simultaneously assess for *C. difficile* and other MDROs. The objectives of this study were to develop rapid qPCR methods to diagnose CDI and other MDRO pathogens from stool DNA samples obtained from hospitalized patients diagnosed with CDI. We randomly collected 25 stool samples from our ongoing CDI surveillance system and extracted stool total bacterial DNA, without any enrichment using a DNA extraction kit. Thereafter, we used 25ng of DNA in qPCRs targeting 16S-DNA and *tcbB* genes for *C. difficile* and vancomycin-resistant gene *vanA*/species-specific DNA sequences of *E. faecalis*/*E. faecium*. The results of qPCRs were calculated as the copy number of target sequences per gram of stool. *C. difficile* was detected from all 25 DNA samples with copy numbers that varied from 10^3 to 2.7x10^8 and copy number of *tcbB* gene that varied from undetectable to 2.9x10^7. Fourteen of 25 samples (56.0%) were positive for both *E. faecalis* and *E. faecium* and nine of 25 samples (36.0%) were positive for *vanA* gene (copy number range: 2.2x10^7 to 6.7x10^10). CDI cases, *Enterococcus* species, and the *vanA* gene was detected from CDI patients using qPCR methods. The method will be useful for rapid and inexpensive surveillance of healthcare-associated infection (HAI) agents.

Email: mjalam@uh.edu

TOXIN A+/B- *CLOSTRIDIOIDES DIFFICILE* ISOLATES DEMONSTRATE MINIMAL VIRULENCE IN ANTIBIOTIC-TREATED MICE

Kirk, L.;[1] Li, Z.;[2] Wellnitz, S.;[2] Liberator, P.;[2] Anderson, A.S.;[2] Bloch, S.C.;[3] Lacy, D.B.;[3] Washington, K.M.;[3] Aronoff, D.M.*[1,3]
[1]Division of Infectious Diseases, Department of Medicine, Vanderbilt University, Nashville, TN USA
[2]Pfizer Vaccine Research and Development, Pearl River, NY USA
[3]Department of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN USA

Background: *Clostridioides difficile* usually encodes 2 large clostridial cytotoxins, toxin A (TcdA) and toxin B (TcdB). Most strains in nature possess intact *tcdA* and *tcdB* genes (and express both TcdA and TcdB). Some express TcdB only ($TcdA^-/TcdB^+$) due to truncations or deletions in *tcdA* and a minority are nontoxigenic. $TcdA^+/TcdB^-$ variants are extremely rare.

Objective: Assess the comparative virulence of $TcdA^+/TcdB^-$ and $TcdA^+/TcdB^+$ strains of *C. difficile* in a mouse model of *C. difficile* infection (CDI).

Methods: 3 $TcdA^+/TcdB^-$ isolates were obtained from patients with CDI. Isolates were tested for TcdA associated toxicity in a Vero cell assay. Female C57/BL6 mice were exposed to cefoperazone prior to gavage with spores of *C. difficile* (range 10^4-10^7 CFU/mouse). Survival, sickness behavior, weight loss, and diarrhea severity were monitored. Toxin titers were estimated using a Vero cell rounding assay with anti-TcdA/TcdB antibody neutralization. Cecal histology and tissue inflammatory mediators via antibody-conjugated bead array were assessed.

Results: Vero cell cytotoxicity elicited by the three TcdA+/TcdB- isolates was neutralized by TcdA mAbs. An inoculum of 10^4 spores of the M7404 $TcdA^+/TcdB^+$ NAP1/027 strain induced severe CDI in antibiotic-treated mice as determined by mortality, sickness behavior, weight loss, and diarrhea, inducing typical histopathological changes of colitis and provoking inflammation. For the three $TcdA^+/TcdB^-$ isolates, only mild colitis with minimal change to tissue inflammatory mediators was observed.

Conclusions: Naturally-occurring $TcdA^+/TcdB^-$ isolates of *C. difficile* demonstrated mild virulence in antibiotic-exposed mice compared to a virulent $TcdA^+/TcdB^+$ strain.

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Email: d.aronoff@vanderbilt.edu

IN VITRO ACTIVITY OF OMADACYCLINE AGAINST SEVEN RIBOTYPES OF *CLOSTRIDIOIDES DIFFICILE*

Begum, K.;* Bassères, E.; Miranda, J.; Lancaster, C.; Gonzales-Luna, A.J.; Carlson, T.; Rashid, T.; Alam, M.J.; Garey, K.W.
University of Houston College of Pharmacy, Houston, TX USA

Omadacycline is a novel aminomethylcycline synthetic antibiotic of the tetracycline class. In clinical trials, omadacycline demonstrated a low rate of *C. difficile* infection (CDI). The objectives of this study were to study the *in vitro* activity of omadacycline and comparators (fidaxomycin, metronidazole, and vancomycin) against a large collection of seven common clinical *C. difficile* ribotypes. In this study, we tested 250 clinical strains from 7 common ribotypes; F001(n=5), F002 (n=56), F014-020(66), F017 (n=8), F027 (n=53), F106 (n=45), and F255 (n=17) collected as part of our ongoing surveillance system. Minimum inhibitory concentration (MIC_{50}, MIC_{90}) for omadacycline, vancomycin, metronidazole, and fidaxomicin were determined after 24 hours incubation using broth microdilution methods according to CLSI guidelines. Omadacycline displayed potent activity against all *C. difficile* ribotypes (Range MIC 50/90: 0.016-0.03125/0.03125-0.05 μg/ml) that was similar to fidaxomicin (0.016-0.03125/0.03125-0.5 μg/ml) and more potent than metronidazole (0.5-2.0/1.0-2.0 μg/ml) or vancomycin (1.0-2.0/1.0-4.0 μg/ml). Increasing vancomycin MIC had no effect on the MIC of omadacycline. Omadacycline displayed potent *in vitro* activity against a large collection of *C. difficile* strains. These results provide a rationale for the low rates of CDI observed in clinical trials and support the further development of omadacycline for the treatment of CDI.

Email: kbegum@central.uh.edu

DEVELOPMENT OF A PHARMACOLOGICAL FORMULATION TO REDUCE *CLOSTRIDIOIDES DIFFICILE* RELAPSE IN A MOUSE MODEL

Castro-Córdova, P.;*[1,2] Mendoza-Leon, M.;[1,2] Díaz-Yáñez, F.;[1,2] Muñoz-Miralles, J.;[1] Gil, F.;[1,2] Paredes-Sabja, D.[1,2]
[1]Millennium Nucleus in the Biology of Intestinal Microbiota, Universidad Andrés Bello, Santiago, Chile
[2]Microbiota-Host Interactions & Clostridia Research Group, Universidad Andrés Bello, Santiago, Chile

Almost 25% of the cases of antibiotic-associated diarrheas are caused by the anaerobic spore-forming bacterium *Clostridioides difficile*, which can cause asymptomatic colonization to pseudomembranous colitis and death in humans. Metronidazole and vancomycin are effective in ~90% of the cases; however, ~30% of the patients manifests relapse of C. difficile infection (RCDI). R-CDI is a major clinical challenge in the treatment of the disease. To date, the only FDAapproved drug shown to reduce R-CDI rates is bezlotoxumab, which is administered via intravenous and in combination with antibiotics.

In this work, we evaluated in a mouse model of R-CDI, the effectivity of a pharmacological formulation that contains antibiotics and an inhibitor of spore persistence. We administered the pharmacological formulation to mice with CDI and observed that the formulation was effective in the treatment of CDI symptoms as vancomycin alone. However, when the treatment was suspended, we observed that during relapse, the treated group with the pharmacological formulation did not lose weight and manifested delayed onset of diarrhea compared with animals treated with vancomycin. During relapse, our formulation was administrated and was similarly effective as vancomycin in resolving CDI symptoms. Moreover, a delay on the onset to diarrhea and colonization during the second episode of relapse was evidenced in mice treated with the pharmacological formulation versus vancomycin alone. Our results demonstrate that the administration of this pharmacological formulation is effective in the treatment of CDI and in reducing the onset of diarrhea during the first and second episodes of relapse.

Email: pablo.castro.cordova@gmail.com

IDENTIFY THE GENETIC CONTRIBUTOR TO *CLOSTRIDIOIDES DIFFICILE* VIRULENCE

Dong, Q.;*[1] Lin, H.;[2] Sia, J.K.;[3] Littmann, E.R.;[2] Mukhopadhyay, S.;[1] Smith, R.C.;[2] Haro, F.;[1] Kohout, C.E.;[2] Pamer, E.G.[1,2]
[1]Departments of Medicine-Infectious Disease, University of Chicago, Chicago, IL USA
[2]Duchossois Family Institute, University of Chicago, Chicago, IL USA
[3]Immunology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY USA

Clostridioides difficile (C. difficile) clinical isolates, those are closely related to the recent emerging hypervirulence strain BI/NAP/027, have identical Pathogenicity Locus (PaLoc) yet cause a wide range of pathogenicity outcomes in mice. This suggests alternative virulence mechanisms are involved. We whole-genome sequenced 54 clinical isolates that belong to the same multi-locus sequencing type family as BI/NAP/027. The presence of identical PaLoc and CdtLoc in each isolate was also confirmed. We have so far characterized 18 strains by both *in vitro* and *in vivo* experiments. No severe growth defect of any isolate was observed upon culturing in liquid broth anaerobically. However, using a mouse model, we were able to classify 8 isolates into high virulence (high mortality, major weight loss and high disease score) and 9 isolates into low virulence phenotype (no mortality, minor weight loss and low disease score). Interestingly, neither the initial colonization or toxin production, measured in fecal pellets, strongly correlates with the virulence phenotype in mice. Preliminary data in most low virulent isolates demonstrated a frequently disruption of collagen-binding adhesin coding genes by the insertion of a potential conjugative element. We are currently applying CRISPR strategies for further validation and mechanistic studies on the gene of interests. Additionally, we discovered one strain that does not cause diarrhea or morbidity in the infected mice, despite high colonization in colon and comparable toxin expression. Our data strongly suggest that even though *C. difficile* toxins are believed to be the major contributors to the bacterial virulence, they do not explain the virulence variations in the hypervirulence strains. Our investigation on the alternative mechanisms will boost our knowledge on hypervirulence *C. difficile* and future clinical evaluation and interventions of *C. difficile* infections.

Email: dongq@uchicago.edu

BUTYRYLATED ASPIRIN HAS ANTIMICROBIAL EFFECTS ON THE GASTROINTESTINAL PATHOGEN, *CLOSTRIDIOIDES DIFFICILE*

Forster, E.R.;*[1,2,4] Yang, X.;[3,4] Shen, A.;[1] Hang, H.C.[3]
[1]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA USA
[2]Graduate School of Biomedical Sciences, Tufts University, Boston, MA USA
[3]Laboratory of Chemical Biology and Microbial Pathogenesis, The Rockefeller University, New York, NY USA
[4]Equal contributors

Short chain fatty acids (SCFAs) generated by the gut microbiota modulate both host immunity and barrier function, as well as virulence of enteric pathogens during infection. Recently, the Hang lab showed that SCFA butyrate inhibits the virulence of *Salmonella typhimurium* by inactivating the SPI-1 transcriptional regulator HilA via acylation. They have further shown that acylating agents, like butyrylated aspirin, can have antimicrobial effects at high concentrations, while at lower concentrations they abolish *S. typhimurium* virulence gene expression, invasion host cells *in vitro*, and disease in mice. Since aspirin can accumulate to high levels in the gut, we tested whether aspirin or its fatty acid derivative, butyrylated aspirin, impacts the growth of *Clostridioides difficile*. We observed that butyrylated aspirin potently inhibited *C. difficile* growth *in vitro* especially when compared to aspirin alone. To elucidate the mechanism of growth inhibition, we identified butyrylated aspirin-binding proteins using an alkyne-butyrylated aspirin probe coupled to proteomics. Interestingly, we found that many of the targets of butyrylated-aspirin are tRNA synthetases, Sec secretion system components, and ribosomal components, suggesting that growth inhibition occurs by interfering with protein synthesis and/or secretion. Here we explore the physiological consequences of inactivating these targets and investigate whether butyrylated aspirin also affects virulence of *C. difficile*. Our strategy of repurposing aspirin and butyrate in combination as a novel compound is a potentially promising means of combating gastrointestinal pathogens.

Email: emily.forster@tufts.edu

MOLECULAR TYPING OF *CLOSTRIDIOIDES DIFFICILE* USING WHOLE GENOME SEQUENCING

Gargis, A.S.;*[1] Vlachos, N.;[1,2] Adamczyk, M.;[1,2] Paulick, A.;[1] McAllister, G.;[1] Korhonen, L.;[1] Guh, A.Y.;[1] Halpin, A.L.;[1] Karlsson, M.;[1] Lutgring, J.D.;[1] EIP CDI Pathogen Group
[1]Centers for Disease Control and Prevention, Atlanta, GA USA
[2] Goldbelt C6, LLC, Chesapeake, VA USA

The CDC performs *Clostridioides difficile* surveillance through the Emerging Infections Program. Whole genome sequencing (WGS) can detect emerging strains and assess genomic diversity. Here we evaluate WGS for molecular characterization of *C. difficile* isolates compared to traditional typing methods.

A subset of 500 isolates representing a random sample of the top 10 ribotypes (RTs) for each year from 2012-2016 (14 RTs total) were selected for WGS (MiSeq, Illumina). PCR-ribotyping and PCR detection of the toxin genes *tcdA*, *tcdB*, *cdtA*, *cdtB*, and deletions in *tcdC* served as reference typing methods. WGS assembly and multilocus sequence typing (MLST) were performed using our in-house QuAISAR-H pipeline. Seven of 14 RTs corresponded to a single sequence type, including RT027 (ST1); RT001_072 (ST3); RT005 (ST6); RT078 (ST11); RT017 (ST37); RT054 (ST43); and RT19 (ST67). The remaining 7 RTs (RT106, RT014, RT015, RT002, RT020, RT056, and RT076) had multiple or overlapping MLSTs, the majority of which represented single- or double-locus variants. All 500 isolates contained *tcdA*, *tcdB*, and *tcdC* using the reference method. WGS detected *tcdA* in 99.6% and *tcdB* in 99.4% of isolates. Discrepancies were due to incomplete assemblies in these regions. RT027 isolates contained distinct sequence diversity in *tcdA*. Complete *tcdA* assembly was not possible for 100/121 (83%) RT027 isolates using short-read WGS data; long-read WGS data will assist with complete assembly. WGS detected *tcdC* in 100% of isolates, including the accurate detection of known deletions. The *cdtA* and *cdtB* genes were present in 142/500 (28.4%) isolates using the reference method. WGS detected *cdtA* and *cdtB* in 144/500 (29%) isolates, with discrepancies due to the lack of detection by reference PCR. Previously described *cdtAB* pseudogenes were detected in 356/500 (71.2%) isolates that did not contain sequences corresponding to *cdtA* or *cdtB*.

C. difficile toxin profiles can be reliably determined using WGS. The MLST was not always predictive of RT; additional phylogenetic analyses will be valuable for assessing genetic relatedness and genomic diversity.

Email: agargis@cdc.gov

EVOLUTIONARY ANALYSIS OF *CLOSTRIDIOIDES DIFFICILE* COLLAGEN-LIKE BCLA EXOSPORIUM PROTEINS

Guerrero-Araya, E.;*[1,2] Paredes-Sabja, D.[1,2]
[1]Millennium Nucleus in the Biology of Intestinal Microbiota, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile
[2]Microbiota-Host Interactions & Clostridia Research Group, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile

Collagen are trimeric proteins composed of repetitive GXY. More than 100 CLPs have been identified in prokaryotes by in silico in analyses, being mostly present in the firmicute group. Bacterial CLPs are typically thought to be surface structures of vegetative cells or bacterial spores. In spore forming bacteria, such as *Clostridioides difficile,* three BclA homologues have been identified, BclA1, BclA2 and BclA3. These CLPs have the following 3 regions: a N-terminal domain (NTD), a central collagen type domain formed by multiple uninterrupted repeats of GXY (CLR) and an C-terminal domain (CTD). Additionally, these CLPs are present in the surface of *C. difficile* spores and previous work has shown that absence of these proteins affect the correct assembly of exosporium, spore resistance properties and cell adhesion. Despite their biological importance, there is currently no comprehensive analysis of their diversities into the 5 phylogenetic MLST clades defined for this species. In addition, there is no evolutionary analysis to address whether positive selective pressure is exerted on the evolution of these exosporium genes. In order to clarify these questions, in this work we investigate the evolution of these 3 CLPs proteins in 2534 publicly available *C. difficile* genomes all of 5 MLST clades by codon-based alignments and phylogenetic analyses to infer their evolutionary history. We show that some of the phylogenetic groups of these proteins are not in agreement with the traditional *C. difficile* classification in clades and recombination signatures were also found. In this work, we show how BclA1, BclA2 and BclA3 are subjected to different evolutionary pressures than the core genome proteins.

Email: biologoenzo@gmail.com

LEPTIN RECEPTOR Q223R POLYMORPHISM INFLUENCES *CLOSTRIDIOIDES DIFFICILE* INFECTION-INDUCED CXCR2 EXPRESSION IN AN IL-1B-DEPENDENT MANNER

Horrigan, O.R.;*[1] Jose, S.;[1] Madan, R.[1,2,3]
[1]Division of Infectious Diseases, University of Cincinnati, Cincinnati, OH USA
[2]Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH USA
[3]Veterans Affairs Medical Center, Cincinnati, OH USA

Clostridiodes difficile infection (CDI) leads to an intense inflammatory response predominated by neutrophils. CDI-induced colonic neutrophilia has a dichotomous role in disease pathogenesis: too many neutrophils are associated with increased tissue damage and high mortality, and neutrophil-depletion also results in high mortality due to transfer of gut bacteria to deeper tissues. We have reported that *C. difficile*-infected mice and humans homozygous for a common mutation in leptin receptor (LEPR, RR genotype) had exaggerated systemic and tissue neutrophilia, which was associated with worse outcomes, when compared to those expressing the wildtype LEPR (QQ genotype). Defining the mechanisms of this differential neutrophilia will identify novel targets to optimize CDI-induced neutrophil responses.

Published studies from our lab show that: (**i**) blocking CXC chemokine receptor 2 (CXCR2) abolishes CDI-induced colonic neutrophilia and (**ii**) neutrophils from *C. difficile*-infected RR mice had higher CXCR2 expression compared to QQ mice. Here, we examined the mechanisms by which CDI upregulates CXCR2 expression using *in vitro* cell culture and flow cytometry assays. Our data reveal that *in vitro* plasma from *C. difficile*-infected mice upregulated neutrophil CXCR2 expression in a dose-dependent manner. Notably, CXCR2 expression was significantly higher on neutrophils exposed to plasma from RR mice, compared to QQ mice, and RR mice had higher circulating Interleukin (IL)-1β. *In vitro*, depletion of IL-1β from RR plasma abolished the increase in CXCR2 upregulation, and recombinant IL-1β increased neutrophil CXCR2 expression. Together, our data indicate that IL-1β-induced CXCR2 upregulation could contribute to higher colonic neutrophilia in RR mice after CDI. Future studies will focus on determining the impact of anti-IL-1β intervention on regulating neutrophilia *in vivo* and defining the molecular mechanisms by which IL-1β upregulates CXCR2 expression.

Email: horrigor@mail.uc.edu

SPECIFC DOMAINS OF THE BCLAS PROTEINS OF *CLOSTRIDOIDES DIFFICILE* SPORES INTERACT WITH CELLULAR COMPONENTS

Inostroza-Mora, A.;*[1,2] Cid-Rojas, F.;[1,2] Paredes-Sabja, D.[1,2]
[1]Millennium Nucleus in the Biology of Intestinal Microbiota, Universidad Andrés Bello, Santiago, Chile
[2]Microbiota-Host Interactions & Clostridia Research Group, Universidad Andrés Bello, Santiago, Chile

The outermost layer of *Clostridioides difficile* spore, named exosporium, has in its surface collagen-like proteins of the BclA family of proteins. The laboratory strain *C. difficile* 630, harbors three orthologous collagen-like exosporium proteins (*bclA1*, *bclA2*, and *bclA3*), by contrast, the most hypervirulent strain *C. difficile* R20291 only encodes two orthologous, *bclA2* and *bclA3*. These proteins are constituted by a central collagen-like region (CLR), formed by multiples repetitions G-X-X, and a carboxy-terminal domain outward-oriented of spore called CTD. Previous work has shown that *C. difficile* spores interact with host cells through projections of the exosporium layer. We have previously shown that *C. difficile* spores interact with extracellular matrix proteins, such fibronectin or vitronectin suggesting that they might interact with the BclA proteins. However, whether these projections are formed by the BclA proteins and how the individual domains of the BclA proteins contributes to spore-host interaction remains unknown. Consequently, in this work, through deletions in each individual domain of the BclA proteins, we evaluate their contribution to assembly of hair-like projections and to interaction with components of the extracellular matrix. Results demonstrate the contribution of each individual domain affect the integrity of the exosporium layer and also contribute to spore resistance to heat and ethanol. Moreover, the contribution of the domains of the BclA proteins to spore adherence is addressed in monolayers of intestinal epithelial Caco-2 cells. Importantly, we address how each individual domain of BclA2 and BclA3 contributes to the interaction of *C. difficile* spores with extracellular matrix proteins. By using recombinant protein of each BclAvariant, we confirm the specificity these interactions. These results provide insight on the role of the exosporium BclA proteins in exosporium biology and how *C. difficile* spores interact with host extracellular matrix molecules.

Email: anainostrozamora@gmail.com

CLOSTRIDIUM DIFFICILE GENOME-SCALE METABOLIC NETWORK ANALYSIS REVEALS CONTEXT-SPECIFIC USAGE OF KNOWN VIRULENCE DETERMINANTS ACROSS MODELS OF INFECTION

Jenior, M.L.;*[1] Powers, D.A.;[1] Dickenson, M.;[1] Tamayo, R.;[4,5] Papin, J.A.[1,2,3]
[1]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA USA
[2]Department of Medicine, Division of Infectious Diseases & International Health, University of Virginia, Charlottesville, VA USA
[3]Department of Biochemistry & Molecular Genetics, University of Virginia, Charlottesville, VA USA
[4]Department of Microbiology & Immunology, University of North Carolina, Chapel Hill, NC USA
[5]Division of Microbial Pathogenesis, University of North Carolina, Chapel Hill, NC USA

Clostridoides (*Clostridium*) *difficile* is a sporulating, toxigenic anaerobe that has become the leading cause of hospital-acquired infection with the prevalence of hypervirulent strains significantly increasing over the last decade. Exposure to antibiotics disrupts the healthy gut microbiota, allowing for *C. difficile* colonization, outgrowth, and disease induction. Unfortunately, antibiotics are the primary means of treatment which allows for approximately one third of patients fall into a cycle of recurrent infection. Previous studies have supported that both colonization and activation of virulence expression are intimately linked to a combination of metabolic signals from the host and members of the gut microbiota. Unfortunately, these interactions remain poorly understood, and it is difficult to determine which specific elements within this environment most strong impact the metabolism of *C. difficile*. More recently, integrative systems biology approaches have provided added context for the integration of omic data and allowed *in silico* modeling of how environmental factors influence metabolic behaviors. In order to understand the behavior of *C. difficile* during infection, we reconstructed metabolic models for strains 630 and R20291 that include key components of core *C. difficile* metabolic strategies and nutrient acquisition systems. We have also developed a novel algorithm for the integration of transcriptomic abundance data into these models that identifies the most cost-effective usage of metabolism that also best reflects the cell's investments into transcription, even when extracellular conditions are unknown. Utilizing these combined *in silico* platforms, we were able to identify distinct substrate utilization *in vitro* with known downstream connections to *C. difficile* virulence activation. Additionally, we uncovered differential usage of core *C. difficile* metabolism across distinct *in vivo* models of infection with varied rates of clearance. These results correlated with the abundance of certain metabolic pathways in the microbiota as well as the measured concentrations of their associated metabolites. Ultimately, this work could result in a predictive platform for the mechanistic connections between the metabolism of the microbiota and pathogen to elucidate novel microbial drivers of recurrent *C. difficile* infection resolution.

Email: mljenior@viginia.edu

OLFACTOMEDIN-4 (OLFM4) EXACERBATES *CLOSTRIDIODES DIFFICILE* INFECTION-INDUCED MORTALITY IN MICE

Jose, S.;*[1] Kassam, A.;[2,3,4] Alder, N.M.;[4,5] Madan, R.[1,6,7]
[1]Division of Infectious Diseases, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH USA
[2]Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, OH USA
[3]Division of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, Cincinnati, OH USA
[4]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH USA
[5]Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH USA
[6]Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH USA
[7]Veterans Affairs Medical Center, Cincinnati, OH USA

Neutrophils are key first-responders in the innate immune response to *C. difficile,* and both the magnitude and duration of neutrophilia affects disease severity and clinical outcomes. Recent literature clearly shows that neutrophils at sites of injury are heterogeneous in nature, with different subpopulations that exhibit a pro-inflammatory, or pro-resolution phenotype. Therefore, a better understanding of the neutrophil diversity after CDI can identify novel pathways that can be targeted to optimize neutrophilic inflammation for better CDI outcomes.

Olfactomedin-4 (OLFM-4) is a granule protein that marks a population of human and mouse neutrophils. Higher number of circulating OLFM4^{+} neutrophils is associated with adverse outcomes in sepsis patients. Here, we examined the role of OLFM4^{+} neutrophils in CDI pathogenesis. We show for the first time that: ***(i)*** complete OLFM4 deficiency (OLFM4$^{-/-}$ mice) results in faster resolution of clinical disease and better survival after CDI; ***(ii)*** OLFM4^{+} cells preferentially aggregated to areas of injured epithelium and their numbers correlated with higher epithelial damage score (Spearman r2=0.6369; p=0.018); ***(iii)*** although mice with severe epithelial damage had more OLFM4^{+} cells compared to those with milder injury, they did not have more *C. difficile* bacteria or toxins; and ***(iv)*** CDI increased recruitment of OLFM4^{+} neutrophils to colonic tissue. Taken together, our data indicate that OLFM4 expression worsens CDI and suggest that OLFM4^{+} neutrophils have a pathogenic role in CDI. Future studies are focused on defining the contribution of OLFM4 expression in neutrophils and non-neutrophil cells using floxed OLFM4$^{-/-}$ mice, and determining the mechanisms by which OLFM4 expression adversely affects CDI outcomes.

Email: josesn@ucmail.uc.edu

DEVELOPMENT, VALIDATION AND CLINICAL VALIDATION OF AN AUTOMATED CELL CYTOTOXICITY NEUTRALIZATION ASSAY FOR DETECTION OF *CLOSTRIDIOIDES DIFFICILE* TOXINS IN STOOLS OF SUSPECTED CDI PATIENTS

Kalina, W.V.;*[1] Nguyen, H.;[1] Tan, C.Y.;[1] Sharma, P.;[1] Wilcox, M.H.;[2] Davies, K.;[2] Jansen, K.U.;[1] Anderson, A.S.;[1] Pride, M.W.[1]
[1]Pfizer Vaccine Research and Development, Pearl River, NY USA
[2]Healthcare Associated Infections Research Group, University of Leeds and Leeds Teaching Hospitals NHS Trust, Leeds, UK

Diagnosis of *Clostridioides difficile* infection is based on clinical findings and microbiological diagnostics. To improve diagnostic accuracy, current US and EU guidelines recommend multistep testing that detects *C. difficile* and toxin in clinically relevant stool samples to confirm active disease. An accepted gold standard to detect *C. difficile* toxins is the cell cytotoxicity neutralization assay (CCNA). While the CCNA is considered one of the most sensitive methods for detecting *C. difficile* toxins, it has limitations that complicates its validation. One such limitation is the subjective interpretation of an analyst to recognize cytopathic effects in cultured cells exposed to a fecal sample containing toxin. To overcome this limitation, an automated CCNA was developed that replaces most human pipetting steps with robotics and incorporates CellTiterGlo for a non-subjective, semiquantitative measure of cell viability. Toxin positivity was based on incorporating two threshold values when evaluating sample dilutions with or without addition of antitoxin antisera: (1) a >70% cell viability threshold with sample containing antitoxin (cells+sample+antitoxin luminescence/cells in media luminescence), and (2) a >1.2-fold difference (cells+sample+antitoxin luminescence/cells+sample luminescence) positivity cutoff where sample results above the cutoff were considered positive. Assay validation demonstrated excellent accuracy, precision, and sample linearity with an LOD of 126.9pg/mL of toxin-B in stool (1pg/mL in media). The positivity cutoff was clinically validated by comparing 322 diarrheal stool sample results to those of a manual CCNA performed at an accredited Reference Laboratory. The automated CCNA demonstrated 96% sensitivity and 100% specificity to the manual CCNA. Stability experiments demonstrated that toxins in diarrheal stool samples were stable for 5 days at temperatures ranging from -20 to 25°C and for 40-months at -80°C. Overall, the automated CCNA is a specific, sensitive, and reproducible tool to support studies on the determination of CDI epidemiology or vaccine efficacy.

Email: warren.kalina@pfizer.com

CLOSTRIDIOIDES DIFFICILE RIBOTYPE DISTRIBUTIONS FOUND IN THE HOSPITAL ENVIRONMENT VERSUS CLINICAL STRAINS

Lancaster, C.;* Gonzales-Luna, A.J.; Begum, K.; Alam, M.J.; Garey, K.W.
University of Houston College of Pharmacy, Houston, TX USA

Purpose: The aim of this study was to assess differences in the distribution of *Clostridioides difficile* ribotypes infecting patients versus those present in the hospital environment.

Methods: A multicenter analysis was conducted using environmental and clinical samples obtained from three Texas hospitals (2017). Leftover stool samples from *C. difficile*-infected patients were collected as part of an ongoing surveillance effort and sent to a central lab for PCR-ribotyping. Investigators collected environmental swabs from each hospital including non-patient care areas, employee shoe bottoms, and patient care areas inside and outside of patient rooms. Environmental swabs were screened for *C. difficile* using CCFA plates and ribotyped.

Results: A total of 1,200 swabs were collected from environmental swabbing (n=400/hospital) along with 137 clinical strains. Of the 1,200 environmental swabs, 310 (25.8%) tested positive for *C. difficile* and ribotypes were obtained for 235 (19.6%) isolates. Of the clinical samples included, 88 (64.2%) had confirmed ribotypes. The most common ribotypes (RTs) from environmental sampling were RT106 (n=46, 19.6%), RT019 (n=36, 15.3%), and RT014-020 (n=11, 4.6%), while the most common clinical ribotypes were RT027 (n=18, 20.5%), RT106 (n=13, 14.8%), and RT014-020 (n=10, 11.4%). Differences were noted in the ribotype distributions between environmental vs clinical isolates overall and within each facility.

Conclusions: This multicenter study demonstrated that many of the same *C. difficile* ribotypes causing infection are present in their surrounding hospital environments, but in differing proportions. Our results imply variables more predictive than ribotype abundance may be influencing strain transmission into patients, but larger studies are warranted.

Email: clancast@centra.uh.edu

PERTURBATION OF THE STOOL MICROBIOME IN THE HAMSTER MODEL OF *CLOSTRIDIOIDES DIFFICILE* INFECTION: THE IMPACT OF ANTIBIOTICS, BACTERIAL CHALLENGE AND IMMUNIZATION

Li, Z.;* Illenberger, D.; Lee, K.; Kanevsky, I.; Anderson, A.S.; Liberator, P.
Pfizer Vaccine Research and Development, Pearl River, New York, USA

Clostridioides (Clostridium) difficile, a Gram-positive, anaerobic, spore-forming, toxin-producing bacillus, is the main cause of nosocomial infectious diarrhea in industrialized countries. Pfizer is developing an investigational bivalent toxoid vaccine to prevent *C. difficile* infection (CDI). Disease is modeled preclinically using a hamster challenge model of CDI. Similar to disease in humans, this model includes antibiotic pretreatment to initiate dysbiosis of the gut microbiome. Hamsters are then challenged with *C. difficile* spores to initiate CDI. Previous studies have shown that hamsters vaccinated with toxin-based vaccines do not succumb to CDI.

The goal of this study was to evaluate the impact of (i) clindamycin treatment, (ii) *C. difficile* spore challenge, and (iii) immunization with a toxin-based vaccine on the gut microbiome in the hamster model of CDI. Stool specimens were collected, total genomic DNA was extracted and the V3-V4 region of the bacterial 16S rRNA gene was amplified from the genomic DNA. Illumina sequencing libraries were prepared and sequenced on the MiSeq platform. Sequence data were analyzed using Qiime2.

Antibiotic treatment had a profound impact on the composition of the hamster stool microbiome. Prior to treatment, the microbiome was dominated by the bacterial phyla *Bacteroidetes* and *Firmicutes*, whereas antibiotics significantly reduced the relative abundance of *Firmicutes* and increased the relative abundance of *Proteobacteria*. Both alpha and beta diversity of the microbiome were significantly altered by antibiotics, but not by immunization. DNA sequence corresponding to the *C. difficile* 16S rRNA gene was detected in the stool microbiome of hamsters treated with antibiotics and challenged with *C. difficile* spores, confirming that the bacterium can colonize in this setting. Compared to immunization and *C. difficile* challenge, antibiotics exerted a much more substantial impact on the stool microbiome.

Email: zhou.li@pfizer.com

ENVIRONMENTAL CONTAMINATION OF WATER WITH *CLOSTRIDIUM DIFFICILE*

Lim, S.C.;*[1] Hain-Saunders, N.M.R.;[1] Riley, T.V.[1,2,3,4]
[1]School of Medical & Health Sciences, Edith Cowan University, Joondalup, WA, Australia
[2]School of Biomedical Sciences, The University of Western Australia, Crawley, WA, Australia
[3]School of Veterinary & Life Sciences, Murdoch University, Murdoch, WA, Australia
[4]Department of Microbiology, PathWest Laboratory Medicine, Nedlands, WA, Australia

Introduction: While *Clostridium difficile* is well-known for causing nosocomial diarrhoea in high-income countries, it is also frequently isolated from a variety of sources outside healthcare settings. With whole-genome sequencing (WGS) showing long-range transmission of *C. difficile* between animals and humans but with no current evidence of an epidemiology link, contaminated environments are suspected of playing a role in the transmission of *C. difficile*. In this study, the prevalence and types of *C. difficile* in rivers, ponds/lakes, estuaries and coastal seawater of Western Australia (WA) was investigated.

Methods: In total, 277 water samples were collected from up to 45km north and 83km south of Perth, the capital of WA. Selective enrichment culture was performed. PCR toxin gene profiling and ribotyping were carried out on isolates.

Results: Seventy-one out of 277 samples (26%) grew *C. difficile*. Pond/lake water had the highest prevalence (48%, 54/112), followed by rivers (23%, 14/61), estuaries (20%, 3/15) and coastal seawater (0%, 0/89). Of the isolates, 23% (18/77) were toxigenic, 17 with toxin gene profile A+B+CDT- and one with the profile A-B+CDT-. *C. difficile*, both toxigenic and non-toxigenic, was widely distributed both north and south of the city. River samples were only positive for *C. difficile* sites upstream from central Perth. Thirty-six ribotypes (RTs) were identified. Of the nine toxigenic RTs, eight were known to cause *C. difficile* infection (CDI) in humans [RTs 014/020 (*n*= 8), 106 (3), 002 (1), 017, (1) 087 (1), 103 (1), QX 076 (1) and QX 454 (1)]. RT 014/020 is also the most common RT recovered from Australian pigs.

Conclusions: *C. difficile* can be found in many different waters around Perth including RT 014/020, one of the most successful lineages causing CDI. Future studies will include using WGS to determine the relatedness of these water isolates to those of animals and humans in WA.

Email: suchen.lim@uwa.edu.au

MURINE INTRARECTAL INSTILLATION OF PURIFIED RECOMBINANT *C. DIFFICILE* TOXINS ENABLES MECHANISTIC STUDIES OF STRUCTURE/FUNCTION RELATIONSHIPS

Markham, N.O.;* Bloch, S.C.; Shupe, J.A.; Washington, M.K.; Coffey, R.J.; Lacy, D.B.
Vanderbilt University Medical Center, Nashville, TN USA

Clostridioides difficile is linked to nearly 500,000 antibiotic-associated diarrheal infections and almost 30,000 deaths per year in the United States. Epidemic strains of *C. difficile* produce toxin A (TcdA) and/or B (TcdB), which directly kill cells and induce an inflammatory response in the colonic mucosa. Hirota, et al. first introduced the murine intrarectal instillation model of infection using purified TcdA/TcdB from VPI and 630 *C. difficile* strains. We present new data expanding this technique by using purified recombinant TcdA and TcdB produced in *Bacillus megaterium,* which allows for the interrogation of how specifically mutated toxins affect the colon. After instillation, mouse colons were processed and stained with H&E for blinded evaluation and scoring by a board-certified GI pathologist. The results show that the amount of TcdA or TcdB needed to produce damage was lower than previously reported. Furthermore, a TcdB mutant (1106K) lacking the ability to form intracellular endosomal pores does not exhibit colonic pathology. Experiments currently in progress are testing the effect of other TcdB functional and structural mutants. We combined TcdA and TcdB in various amounts to show a substantial damaging effect of only small amounts of TcdA. Tissue sections were also immunostained using antibodies against Ly6G, MPO, or F4/80. The acute inflammatory response was quantified using semi-automated imaging analysis to remove the inherent observer bias and categorical variables used with traditional histopathological scoring. These staining patterns were also compared with colonic tissue from mice after *C. difficile* spore gavage or with human colonic tissue from a patient with *C. difficile* infection. In summary, we have adapted the intrarectal instillation model to show that purified recombinant TcdA and TcdB are potent reagents with the flexibility to better understand structure/function relationships in pathogenesis. Furthermore, we have applied additional imaging analysis to improve the interpretation and comparison of the acute inflammatory response to *C. difficile* toxins.

Email: nick.markham@vumc.org

HIGH-LEVEL CHARACTERIZATION OF *CLOSTRIDIOIDES DIFFICILE* ISOLATES FOR THE CDC & FDA ANTIBIOTIC RESISTANCE ISOLATE BANK

Paulick, A.;* Adamczyk, M.; Vlachos, N.; McAllister, G.; Machado, M.; Korhonen, L.; Guh, A.Y.; Halpin, A.L.; Karlsson, M.; Rasheed, J.K.; Lutgring, J.D.; Gargis, A.S.; EIP CDI Pathogen Group
Centers for Disease Control and Prevention, Atlanta, GA USA

Clostridioides difficile infection (CDI) is among the most common healthcare-associated infections; however, there is a lack of well-characterized publicly available isolates representing the current landscape of CDI in the United States. A diverse panel of 30 *C. difficile* isolates collected through the Emerging Infections Program (EIP) in 2016 was selected to undergo high-level characterization, including antimicrobial susceptibility testing (AST) and whole genome sequencing (WGS). Here, we describe the characterization of these isolates and announce their availability in the CDC & FDA Antibiotic Resistance Isolate Bank (AR Bank).

Among isolates characterized by PCR-ribotyping in 2016, the most prevalent ribotypes (RTs) were 027, 106, 002, 014, 020, 015, 056, 054, 019, and 078. A subset of 30 community- and healthcare-associated isolates representing the top 10 RTs were selected from 10 EIP sites (CA, CO, CT, GA, MD, MN, NM, NY, OR, TN). All isolates carried toxin genes *tcdA* and *tcdB*, and 20/30 (67%) contained a wildtype *tcdC* sequence by PCR detection. Ten isolates contained binary toxin genes *cdtA* and *cdtB*. Agar dilution AST was performed for ceftriaxone, clindamycin, meropenem, metronidazole, moxifloxacin, and vancomycin. Of the drugs tested, only vancomycin and metronidazole are clinically indicated for treatment of CDI; no isolates displayed elevated MICs to vancomycin based on the CLSI epidemiological cut-off value, and all isolates were susceptible to metronidazole. Isolates were sequenced and known resistance mechanisms identified. Five isolates harbored an *erm* gene, 2 carried the *cfr* gene, and 7 harbored an amino acid substitution (Thr82Ile) in the *gyrA* gene which is associated with fluoroquinolone resistance.

This panel is the first publicly available resource for highly characterized *C. difficile* isolates, including AST and WGS data. Additional panels may be added to the AR Bank to provide a growing source of contemporary isolates for use by the community as reference materials.

Email: wnk1@cdc.gov

BILE SALT ANALOGS ACT AS ANTI-GERMINANTS FOR *CLOSTRIDIOIDES* DIFFICILE INFECTION (CDI) PROPHYLAXIS

Phan, J.R.;*[1] Do, D.M.;[1] Truong, M.C.;[1] Ngo, C.;[1] Mata, T.V.;[1] Phan, J.H.;[1] Sharma, S.K.;[2] Firestine, S.M.;[2] Abel-Santos, E.[1]
[1]University of Nevada, Las Vegas Department of Chemistry & Biochemistry Las Vegas, NV USA
[2]Wayne State University, Department of Pharmaceutical Sciences, Detroit, MI USA

Clostridioides [*Clostridium*] *difficile* infection (CDI) is responsible for the majority of nosocomial antibiotic-associated diarrhea. With rises in both hospital- and community-acquired CDI incidences due to the emergence of hypervirulent strains, CDI recurrences can reach up to 25%. Thus, standard treatments are rendered less effective, making new methods of prevention more critical. Since *C. difficile* spore germination is a necessary step for CDI establishment, methods that target this process could serve for infection prophylaxis. *C. difficile* spore germination is promoted by the bile salt taurocholate. Previously, CamSA, a synthetic bile salt analog of taurocholate, was found to be a more potent germination inhibitor than the endogenously-present inhibitor chenodeoxycholate when tested against strain 630. CamSA also protected mice challenged with strain 630 spores. In the present study, multiple bile salt analog compounds have been found to be much stronger germination inhibitors than CamSA against the strain 630 spores. These compounds were also shown to be effective anti-germinants against seven other strains representing different *C. difficile* ribotypes, including hypervirulent strain R20291. Germination assays demonstrated that these compounds inhibited spore germination in each tested *C. difficile* strain with IC_{50} values of micromolar concentrations. *In vivo*, a select few of these analogs were also effective at reducing infection signs in rodent CDI models. One compound, SKS-VI-07C, was capable of reducing, delaying, or preventing CDI symptoms in both hamsters and mice infected with *C. difficile* spores. The results of this study illustrate the general patterns of disease and inhibitory abilities of bile salt analogs. From these explorations, bile salt analogs may have the potential to serve in the prophylactic treatment of CDI in antibiotic-treated patients.

Email: phanj2@unlv.nevada.edu

ROLE OF BCLA2 AND BCLA3 IN THE ASSEMBLY OF THE EXOSPORIUM LAYER OF *CLOSTRIDIUM DIFFICILE* SPORE

Pizarro-Guajardo, M.;*[1,2] Paredes-Sabja, D.[1,2]
[1]Millennium Nucleus in the Biology of Intestinal Microbiota, Universidad Andrés Bello, Santiago, Chile
[2]Microbiota-Host Interactions & Clostridia Research Group, Universidad Andrés Bello, Santiago, Chile

C. difficile is a Gram-positive, endospore-forming and anaerobic bacterium responsible for diarrhea associated to antibiotics and dysbiosis in gut microbiota. *C. difficile* spore is the morphotype responsible for transmission, infection, persistence in patient gut, and therefore, is also responsible for the development of recurrence that affects to 25 – 65 % of patients.

The external layer of spore, exosporium, contains surface molecules that contact with host, ephitelium, and immune cells. In the exosporium, nearly 21 structural proteins has been described, among them, collagen-like proteins *bclA2* and *bclA3*. Genetic arrangement of *bclA2* and *bclA3* in R20291 shows that both genes are dependent promoters that contain consensus sequence for sigma K and sigma E. While *bclA2* is monocistronic, *bclA3* is the second gene in a bisistronic operon, and expression is driven by an additionaly sigma K consensus sequence immediately upstream of *bclA3*. In this work, we evaluate the role of BclA2 and BclA3 in the formation of the hair-like extensions in the spore and in pathogenesis of *C. difficile*. The implication of BclA2 and BclA3 on the formation of the hair-like extension was assessed by immunogold labelling. The contribution of BclA2 and BclA3 to spore adherence to epithelial cells was assessed in monolayers of intestinal epithelial cells of Caco-2 cells. We also assessed the effect of changing the temporal expression of BclA2 and BclA3 in exosporium formation and spore properties. Collectively, these results provide new evidence of the role of BclA proteins in the assembly of the exosporium layer of *C. difficile* spores.

Email: marjorie.pizarrog@gmail.com

COMPARATIVE ANALYSIS IN ANIMAL MODELS REVEALS DIFFERENCE IN INFLAMMATORY RESPONSES AND VIRULENT POTENTIAL OF FOUR STS FROM *CLOSTRIDIUM DIFFICILE* MLST CLADE 2

Orozco-Aguilar, J.;[1,2] Alfaro-Alarcón, A.;[3] Acuña-Amador, L.;[4]
Chaves-Olarte, E.;[4] Rodríguez, C.;[4] Quesada-Gómez, C.*[1,4]
[1]Laboratorio de Ensayos Biológicos (LEBi), Universidad de Costa Rica, San José, Costa Rica
[2]Facultad de Farmacia, Universidad de Costa Rica, San José, Costa Rica
[3]Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica
[4]Centro de Investigación en Enfermedades Tropicales and Facultad de Microbiología, Universidad de Costa Rica San José, Costa Rica

Clostridium difficile infection cases have been increasing in incidence and severity. The global dissemination of epidemic NAP1/RT027/ST01 strain is a relevant epidemiological issue since it is associated with higher mortality rates and antibiotic-associated diarrhea outbreaks. This strain has been classified by MLST in the so-called hypervirulent clade 2, along with other sequence types (ST) which are not epidemic. This study presents the pathogenic and virulent potential of five clinical isolates of different sequences types (ST) from clade 2. To this end, we determined the pro-inflammatory and lethal activity using murine ileal loop and hamster's infection models for strains of ST01, 41, 67, and 252. The animal models showed differences in pro-inflammatory capacity and virulence. The supernatants from ST01 strain produced a higher pro-inflammatory responses and epithelial damage in the mouse model than did other strains. The increased virulence of ST01 strain was confirmed by mortality rates and biochemical blood markers in the hamster model. Furthermore, a heterogeneity in the pathogenic potential was demonstrated, since strains from ST67 and ST41 showed stronger reactions in both models than did ST252. These results highlight that there are differences in the virulent potential of the studied STs, although these belong to the hypervirulent clade 2. This suggests that combination of diverse virulence factors in each ST explain our results and these factors are not included in the MLST for the genetic classification of *C. difficile*.

Email: carlos.quesada@ucr.ac.cr

STRAIN DEPENDENT INHIBITION OF *CLOSTRIDIOIDES DIFFICILE* BY COMMENSAL *CLOSTRIDIA* ENCODING THE BILE ACID INDUCIBLE (*BAI*) OPERON

Reed, A.D.;*[1] Nethery, M.A.;[2] Barrangou, R.;[2] Stewart, A.;[3] Theriot, C.M.[1]
[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC USA
[2]Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC USA
[3]Molecular Education, Technology and Research Innovation Center, North Carolina State University, Raleigh, NC USA

Clostridioides difficile is one of the leading causes of antibiotic-associated diarrhea. Gut microbiota derived secondary bile acids and commensal *Clostridia,* such as *C. scindens,* that encode the bile acid inducible (*bai*) operon, are associated with protection from CDI, although the mechanism is not known. Thus, we hypothesized that commensal *Clostridia* were able to inhibit *C. difficile* through the production of inhibitory secondary bile acids, such as deoxycholate (DCA), from primary bile acid cholate (CA). Supernatants from commensal *Clostridia* grown with different concentrations of CA were added to *C. difficile* cultures to assess inhibition, and LC/MS was performed to assess the concentration of CA and DCA. Expression of selected genes from the *bai* operon was assessed through qRT-PCR. When *C. scindens* ATCC and VPI were grown in the presence of 2.5 mM CA, 1.96 mM DCA was produced and *C. difficile* growth significantly decreased. *C. hiranonis* only produced 0.78 mM DCA when supplemented with 2.5 mM CA, while *C. hylemonae* did not produce any DCA. Neither strain grown with CA was able to inhibit *C. difficile* growth. Consistent with the LC/MS results, expression of *bai* operon genes increased in *C. scindens* ATCC, *C. scindens* VPI and *C. hiranonis,* but not *C. hylemonae.* Competition for nutrients was also examined using an *in vitro* competition assay. *C. difficile* outcompeted all four commensals in rich media. The ability of commensal *Clostridia* that encode the *bai* operon to inhibit *C. difficile in vitro* in the presence of CA is strain dependent and correlates with the amount of DCA produced. Understanding the relationship between commensal *Clostridia* and the pathogen *C. difficile* in the gut is vital for designing targeted bacterial therapeutics.

Email: adreed2@ncsu.edu

QUANTITATIVE ANALYSIS OF *CLOSTRIDIOIDES DIFFICILE* SINGLE-CELL GROWTH DYNAMICS USING TIME-LAPSE MICROSCOPY

Ribis, J.W.;*[1,2] Aldridge, B.;[1] El Meouche, I.;[3] Dunlop, M.;[3] Shen, A.[1]
[1]Tufts University School of Medicine, Boston MA USA
[2]Tufts University Graduate School of Biomedical Sciences, Boston MA USA
[3]Boston University School of Engineering, Boston MA USA

Clostridioides difficile is a spore-forming anaerobe that is a leading cause of nosocomial infection. While *C. difficile's* ability to produce potent toxins allow it to cause disease, little is understood about the factors that allow *C. difficile* to grow and survive in the gut environment. *C. difficile* exhibits considerable phenotypic heterogeneity within a clonal population, likely as a strategy to 'bet-hedge' and promote survival in rapidly changing conditions. Unfortunately, long-term microscopic analyses to test this hypothesis have been challenging, since oxygen is toxic to *C. difficile*. We have developed a simple time-lapse imaging method that allows for automated growth measurements of individual *C. difficile* cells. We recently applied this method to study the single-cell growth dynamics of clinical isolates spanning multiple clades and ribotypes. These analyses revealed that Clade B strains grow faster (~25%) and produce ~40% longer cells than Clade A strains. We have also used this system to study *C. difficile* growth during exposure to physiologically relevant stressors such as secondary bile acids. We observed increased heterogeneity in growth across all strains due to the emergence of distinct sub-populations of cells with distinct growth parameters. We have also applied our system to analyze *C. difficile* germination and outgrowth at the single-spore level. Last, we have applied novel anaerobic fluorescent reporters to identify and track the fate of *C. difficile* subpopulations with different transcriptional profiles over time. Since our approach uses only readily available commercial reagents, it should also be broadly applicable to study the growth dynamics of a variety of anaerobes.

Email: john.ribis@tufts.edu

CHARACTERIZING THE MOLECULAR INTERACTIONS BETWEEN *CLOSTRIDIOIDES DIFFICILE* AND *ENTEROCOCCUS FAECALIS*

Smith, A.B.;*[1,2] Hart, J.L.;[2] Keenan, O.N.;[2] Rangel, P.C.;[2] Specker, J.T.;[3] Prentice, B.M.;[3] Zackular, J.P.[1,2]
[1]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA US
[2]Division of Protective Immunity, Children's Hospital of Philadelphia, Philadelphia, PA USA
[3]Department of Chemistry, University of Florida, Gainesville, FL USA

Clostridioides difficile is the most frequently reported hospital-acquired pathogen in the United States and an urgent public health threat worldwide. The primary risk factor for *C. difficile* infection (CDI) is antibiotic use, which reduces colonization resistance to *C. difficile* by perturbing the resident gut microbiota. Despite the well-established link between the gut microbiota and susceptibility to *C. difficile*, little work has been done to explore the impact of *C. difficile*-microbiota interactions during infection. Polymicrobial interactions can be attributed to the development of numerous infections and cross talk between pathogens is associated with exacerbation of disease. Here, we show that the antibiotic-resistant *Enterococci* are some of the most highly abundant members of the microbiota in the *C. difficile*-infected gut. Using an *in vitro* co-culturing system, we demonstrate that *Enterococcus* dramatically alters the morphology, metabolic state, and virulence of *C. difficile*. Our preliminary data suggests that metabolite cross-feeding plays a central role in these interactions, leading to increased *C. difficile* toxin production and virulence. Together, these data suggest that antibiotic resistant members of the microbiota may play a previously unappreciated role in *C. difficile* behavior and virulence and could provide a novel therapeutic target for CDI.

Email: alexsmith5487@gmail.com

DEFINING THE PROTECTIVE ROLE OF PROSTAGLANDINS DURING *CLOSTRIDIOIDES DIFFICILE* INFECTION

Soto Ocaña, J.S.;*[1,2] Aronoff, D.A.;[3] Zackular, J.P.[1,2]
[1]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA USA
[2]Division of Protective Immunity, Children Hospital of Philadelphia, Philadelphia, PA USA
[3]Division of Infectious Diseases, Vanderbilt University Medical Center, Nashville, TN USA

Clostridioides difficile is the most commonly reported nosocomial pathogen and is an urgent public health threat. This Gram-positive, spore-forming anaerobic bacterium colonizes the colon, causing a wide range of symptoms varying in severity from mild diarrhea to toxic megacolon and/or death. The factors responsible for this broad spectrum of disease are largely unknown, but likely include host, microbiota, and environmental factors. Prostaglandins (PGs) are important lipid mediators produced by the host that protect the gastrointestinal tract from damage through maintenance of mucosal integrity and promotion of wound healing. We recently demonstrated that prostaglandins are able to protect against severe *C. difficile* infection (CDI). Mice infected with *C. difficile* and treated with misoprostol, an FDA approved prostaglandin E_1 (PGE_1) analog showed increased survival. We observed that this effect was due to maintenance of tight junction integrity and recovery of the epithelial cell barrier. Additionally, mice infected *C. difficile* and treated with non-steroidal anti-inflammatory drugs (NSAIDs), a group of drugs that inhibit PGs synthesis, showed dramatic exacerbation of disease and disruption of the epithelial cell barrier. To dissect the mechanism of prostaglandin-mediated protection during CDI, I have developed and *in vitro* system using Caco-2 intestinal epithelial cells. When endogenous prostaglandin production is blocked in Caco-2 using NSAIDs, cellular cytotoxicity increases. In the gut, prostaglandin E_2 (PGE_2) is able to bind four different types of EP receptor ($EP_{1\text{-}4}$). Signaling of PG_2 through EP_4 receptor induces different signaling pathways involved in mucin production, cytoprotection and motility. Thus, I hypothesize that NSAID-mediated inhibition of prostaglandins are preventing PG_2 signaling through EP_4 which might be responsible of protecting the intestinal epithelial cells from *C. difficile* detrimental effects.

Email: jsocana@pennmedicine.upenn.edu

A NOVEL ENDOPEPTIDASE (R20291_0971) PLAYS PLEIOTROPIC ROLES IN *CLOSTRIDIOIDES DIFFICILE*

Zhu, D.;[1] Lamabadu Warnakulasuriya Patabendige, H.M.;[1] He, Y.;[2] Sun, X.*[1]
[1]Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL USA
[2]Department of Microbiology and Immunology, and Center for Computational Medicine and Bioinformatics, Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI USA

Clostridioides difficile is a Gram-positive, spore-forming, toxin-producing anaerobe and can cause nosocomial antibiotic-associated intestinal disease. While production of toxin A (TcdA) and toxin B (TcdB) contribute to the main pathogenesis of *C. difficile*, the mechanism of TcdA and TcdB secretion from the pathogen are still unclear. In this study, we applied the Vaxign reverse vaccinology tool, and characterized a new endopeptidase (CDR20291_0971). R20291Δ0971 generated with CRISPR-AsCpfI exhibited significantly delayed cell autolysis and permeability decrease compared to the wild type strain. Meanwhile, biofilm, germination and sporulation of mutant also showed significant decrease. Moreover, 0971 deletion impaired TcdA and TcdB secretion in stationary and late phase of cell growth which decreased cytotoxicity and fitness over the parent strain in a mouse infection model. Furthermore, we detected cell viability through LDH cytotoxicity detection and live-dead cell staining analysis, and confirmed the decreased permeability and increased cell viability of the R20291Δ0971. Finally, we purified a recombinant 0971 protein and showed that it has lytic transglycosylase activity. Thus, endopeptidase 0971 protein is involved in cell wall integrity and viability, which could comediate toxin release of R20291 with holing-like TcdE protein. Our data also suggest that the endopeptidase 0971 is an attractive target for CDI therapeutics and prophylactics.

Email: sun5@usf.edu

Fusobacteria

Fusobacteria

DEVELOPING A SEROTYPING SCHEME BASED UPON *FUSOBACTERIUM NUCLEATUM* LIPOPOLYSACCHARIDE O-ANTIGENS

Cairns, C.M.; St. Michael, F.; Fleming, P.; Vinogradov, E.; Cox, A.D.*
Human Health Therapeutics Research Centre, National Research Council, Ottawa, ON, Canada

Fusobacterium nucleatum (Fn) is becoming increasingly recognised as an emerging pathogen, especially when it escapes its normal home of the mouth. Recently, Fn has been gaining attention as a potential factor for exacerbating colorectal cancer and is strongly linked with pregnancy complications including pre-term and still births. Little is known about the virulence factors of this organism, and thus, we have initiated studies to examine the bacterium's surface glycochemistry. Furthermore, taxonomically, Fn is a challenging organism to classify with at least five subgroups of Fn known (*animalis*, *fusiforme*, *nucleatum*, *polymorphum*, and *vincentii*).

In an effort to characterise the surface carbohydrates of Fn, coupled with a view to improving the typing tools available to characterise this organism, this study has set out to investigate the structure of the lipopolysaccharide (LPS) O-antigens of Fn and to develop immunological tools in order to establish a serotyping scheme to classify the different strains of Fn based upon their O-antigen structures.

This study has combined several technologies including large scale anaerobic fermentation, analytical structural chemistry including nuclear magnetic resonance spectroscopy (NMR), and monoclonal antibody (mAb) technologies.

Our studies so far have isolated and characterised LPS O-antigen structures from over ten strains and developed mAbs to the majority of them. A variety of O-antigen structures have been identified and although several structural similarities have been observed, the structural variation has so far limited our ability to develop a serotyping scheme.

Several unique sugars have been identified, including several variants of nonulosonic acid from the simplest sialic acid to much more complex nine carbon sugars along with the common presence of 6-deoxy-N-acetyl hexosamines.

Further studies will be performed with additional strains to facilitate further development of the serotyping scheme.

Email: andrew.cox@nrc-cnrc.gc.ca

CASE REPORT: BRAIN ABSCESS SECONDARY TO *FUSOBACTERIUM NUCLEATUM* MIMICKING *NOCARDIA* WITH ANTIBIOTIC EFFECT

Cumagun, P.M.;* Marrazzo, J.
Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL USA

Background: Fusobacteria are nonmotile, Gram-negative obligate anaerobic bacilli that are usually a part of the normal flora of the human oropharynx, upper respiratory tract, gastrointestinal tract, and genitourinary tract. *Fusobacterium nucleatum* can cause a broad array of diseases, most commonly pharyngitis and less commonly, a more disseminated disease process such as an odontogenic infection that may extend to a brain abscess.

Case presentation: We report a case of a previously healthy 71-year-old Caucasian male who presented with new onset seizure and was found to have a ruptured intracerebral abscess resulting in ventriculitis and bacterial meningitis. He was treated presumptively with IV Vancomycin, Ceftriaxone, Ampicillin, and Acyclovir at an outside hospital, where cerebrospinal fluid (CSF) cultures were without growth. At our institution, a repeat CSF gram stain showed Gram positive branching rods concerning for *Nocardia* species with a morphology consistent with antibiotic effect; CSF cultures remained negative. He was presumptively treated for *Nocardia* with trimethoprim-sulfamethoxazole and imipenem and improved over the course of several days. However, the diagnosis of *Fusobacterium nucleatum* was finally made based on a broad-range 16S ribosomal RNA gene polymerase chain reaction (PCR), altering the patient's treatment and duration course. He was treated with a six-week course of imipenem for *Fusobacterium* and recovered well with only mild cognitive changes.

Conclusion: This case report highlights the importance of considering *Fusobacterium* species as a cause of brain abscesses. Furthermore, this case also highlights the role of 16s PCR in diagnosing culture negative brain abscesses and emphasizes how this diagnostic tool can alter management.

Email: pcumagun@uabmc.edu

COLONIZATION DYNAMICS AND TUMORIGENIC POTENTIAL OF COLORECTAL CANCER-DERIVED *FUSOBACTERIUM NUCLEATUM* SUBSPECIES IN MURINE MODELS

Domingue, J.C.;*[1] Queen, J.;[1] Drewes, J.L.;[1] Bullman, S.;[2] Stevens, C.;[1] Ding, H.;[1] Housseau, F.;[3,4] Sears, C.L.[1,3,4]
[1]Infectious Diseases, Johns Hopkins University, Baltimore, MD USA
[2]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[3]Oncology, Johns Hopkins University, Baltimore, MD USA
[4]Bloomberg-Kimmel Institute, Johns Hopkins University, Baltimore, MD USA

Studies have shown that *Fusobacterium nucleatum* (Fn) is prevalent in human colorectal cancers (CRC) and can induce colonic tumors in mice. However, these mouse studies required frequent gavages and lacked assessment of Fn colonization. We examined the colonization dynamics and tumorigenic potential of CRC-derived Fn subspecies (ssp.) *animalis (Fn-a), nucleatum (Fn-n), vincentii (Fn-v),* and *polymorphum (Fn-p)* using C57BL/6 wildtype (WT) and $Apc^{Min\Delta 716/+}$ (Min) mice under germ-free (GF) and specific pathogen free (SPF) conditions. To test whether Fn ssp. would differentially colonize the gut of GF mice, we first inoculated WT mice once and harvested at 2 weeks (wks) for fecal DNA (fDNA) analysis by Fn-specific 16S rRNA qPCR. All Fn ssp. colonized (10^5 Fn copies/5ng fDNA), except for *Fn-p* (<10^1 Fn copies/5ng fDNA). Despite having lower colonization, *Fn-p* induced equivalent levels of IL-6 and IL-1b as the other Fn ssp. We then assessed colonic tumorigenesis of GF Mins orally gavaged weekly (for 4wks) with *Fn-a, Fn-v, or Fn-p.* Similar to findings in WT GF mice, *Fn-a* and *Fn-v* readily colonized after the initial gavage, whereas *Fn-p* required repeated gavage. Despite robust colonization by all Fn ssp. by week 4 (10^7 Fn copies/5ng fDNA), there were few colonic tumors at harvest (11wks; *Fn-a*: 0; *Fn-v*: 1/5 mice=1 tumor; *Fn-p*: 2/4 mice=1 tumor). Using the same colonization protocol, we evaluated colonization in WT SPF mice that received oral cefoxitin for 2 days prior to initial inoculation followed by gentamicin throughout the experiment. This resulted in moderate colonization by *Fn-v* ($5x10^2$ Fn copies/5ng fDNA), but not by other Fn ssp. (<$5x10^1$ Fn copies/5ng fDNA); further, colonization was lost by 2wks after the final gavage. Our findings suggest that Fn ssp. exhibit different gut colonization potential and induce minimal colon tumorigenesis in GF Mins. Thus, we hypothesize that Fn ssp. are secondary invaders of colon tumors potentially facilitated by a changing tumor microenvironment.

Email: jdoming9@jhmi.edu

CHARACTERIZATION OF A NOVEL FUSOBACTERIUM SPECIES ISOLATED FROM A HUMAN COLON TUMOR

Eisele, Y.;*[1,2] Baryiames, A.;[1] Wu H.;[1] LaCourse, K.;[1] Johnston, C.D.;[1] Bullman, S.[1]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]Technical University of Munich, School of Medicine, Munich, Germany

Fusobacteria are Gram negative, anaerobic human commensals and pathogens associated with a wide spectrum of diseases, including periodontitis and preterm birth. Recent studies have consistently demonstrated an enrichment of *Fusobacterium nucleatum* in colorectal adenocarcinoma compared to adjacent normal tissue.

Here we report the isolation and characterization of a novel Fusobacterium species (Fuso_Nov_143CP) from primary colon adenocarcinoma tissue of a female patient with microsatellite-stable, stage 3, right-side colon cancer.

PacBio whole genome sequencing of Fuso_Nov_143CP revealed a genome size of 2.35Mb and a G+C content of 30 mol%. Average nucleotide identity values confirmed its position within the Fusobacterium genus and novelty at the species level. At the 16S rRNA gene level, *F. perfoetens* is its closest related species. Preliminary metagenomic analyses demonstrate this novel species is present in >10% of human colorectal tumors and in 4-13% of fecal or tissue specimens from healthy and inflammatory bowel disease patients. Fuso_Nov_143CP forms distinct round, opaque viscous colonies with white central indentions following 48hrs anaerobic culture. Transmission electron microscopy (TEM) reveals this novel species is a short rod with an average length of 1.6μm and width of 1.2μm.

Metabolic testing demonstrated Fuso_Nov_143CP's ability to ferment lactose, esculin, mannose, and raffinose, and its possession of alpha-galactosidase, an enzyme also utilized for conversion of human blood group B to 0.

Similar to *F. nucleatum*, Fuso_Nov_143CP is bile-sensitive, does not reduce nitrate and is susceptible to Metronidazole, Colistin and Penicillin G. Short-term co-culture of Fuso_Nov_143CP with HCT-116 cells led to a moderate increase in the cytokine IL-8. However, TEM under the same conditions did not unveil adhesion or invasion to colon cancer cells.

Our study sheds light on a novel *Fusobacterium* species, that appears to be a prevalent member of the human lower gastrointestinal microbiota. However, its role in human health and disease requires further investigation.

Email: yeisele@fredhutch.org

FUSOBACTERIUM NUCLEATUM INTERACTS WITH *CLOSTRIDIUM DIFFICILE* IN THE INTESTINAL MUCUS LAYER

Engevik, M.;*[1,2] Danhof, H.A.;[3] Auchtung, J.;[3] Britton, R.;[3] Versalovic, J.[1,2]
[1]Department of Pathology & Immunology, Baylor College of Medicine, Houston, TX USA
[2]Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital, Houston, TX USA
[3]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX USA

Background*: Clostridium difficile* infection (CDI) is a major nosocomial disease with limited treatment options. Therefore, understanding the mechanisms of CDI and developing novel therapeutics is a high priority. *C. difficile* has been observed in the MUC2 mucus layer of mice and patients, indicating that *C. difficile* may establish a mucus-related niche and interact with members of the mucus-associated microbiome. We hypothesized that certain mucus-associated microbes would promote *C. difficile* colonization and biofilm formation.

Methods & Results: To address this hypothesis, we utilized a human centered approach. We found that *C. difficile* adhered to O-glycan structures on human MUC2 using mucin-producing cells lines and human colonoid (organoid) monolayers. To identify other microbes interacting with *C. difficile*, we used bioreactors with stool and human MUC2-coated coverslips. 16S rRNA sequencing of MUC2-coverslips revealed a unique biofilm profile with substantial co-colonization with *Fusobacterium*. Chemotaxis assays revealed that motile *C. diffiicle* was chemoattracted to *Fusobacterium nucleatum* subspecies *nucleatum*, *polymorphum*, and *animalis*. We also observed that *C. difficile* co-aggregated with all *F. nucleatum* subspecies; an effect that was dependent on *F. nucleatum* adhesin RadD and *C. difficile* flagella. Minimal aggregation was observed with other members of the gut microbiota. Addition of *F. nucleatum* enhanced *C. difficile* biofilm formation and these biofilms were more resistant to chemical and antibiotic disruption. RNAseq data revealed significant changes in *C. difficile* surface adhesion, ethanolamine utilization and antimicrobial resistance genes following exposure to *F. nucleatum* metabolites. Finally, publicly available datasets and patient stool samples revealed that a subset of patients with *C. difficile* infection exhibited high levels of *Fusobacterium* OTUs. *C. difficile* and *F. nucleatum* were found to co-localize by FISH in CDI positive adult stool and surgical resections.

Conclusions: Together, these data demonstrate the unique role of mucus-associated bacteria such as *F. nucleatum* in enhancing *C. difficile* mucus-associated biofilms and promoting antimicrobial resistance.

Email: melinda.engevik@bcm.edu

IDENTIFICATION OF FUSOBACTEIUM SUBSPECIES BY MATRIX-ASSISTED LASER DESORPTION IONIZATION–TIME OF FLIGHT MASS SPECTROMETRY

Hayashi, M.;* Hanh, V.T.M.; Yokoyama, S.; Tanaka, K.
Division of Anaerobe Research, Life Science Research Center, Gifu University, Gifu, Japan

Background: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been widely used in clinical microbial diagnosis in the last decade. However, several studies have indicated that MALDI-TOF MS showed some limitations in identifying anaerobic bacteria, including similar spectra patterns between different species, closely related species variation of protein spectra, and the limited spectra database.
In present study, we evaluated the use of MALDI-TOF MS for the identification of *Fusobacterium nucleatum* at the subspecies level.

Methods: Fourty-one *F. nucleatum* isolates comprising the type strains and clinical isolates of the five subspecies (16 strains of *animalis*, 7 strains of *nucleatum*, 4 strains of *polymorphum*, 3 strains of *fusiforme* and 11 strains of *vincentii*) were identified by MALDI Biotyper with version 3.1 database. 16S rRNA gene sequencing was used as reference method for accuracy in the identification.

Results & Discussion: MALDI-TOF MS gave high confidence identification results for 41 *F. nucleatum* strains. MALDI-TOF MS with Bruker database further correctly identified 39 of 41 *F. nucleatum* clinical isolates to the subspecies level.

In the cluster analysis by MALDI-TOF MS, strains of subsp. *animalis*, subsp. *nucleatum* and subsp. *polymorphum* formed one cluster within each subspecies group. However, strains of subsp. *fusiforme* and subsp. *vincentii* were distributed within the same cluster. Furthermore, several small clusters were found in the clusters for subsp. *animalis*, subsp. *nucleatum* and subsp. *polymorphum*. In our study, no association between the source of strains and those small clusters was recognized. MALDI-TOF MS with newly database can be used in clinical microbiology field to identify *F. nucleatum* at the subspecies level.

Email: masah@gifu-u.ac.jp

FUSOBACTERIUM SIGNALLING IN DISEASE-ASSOCIATED ORAL BIOFILMS

Kuehne, S.A.;*[1,2] Muchova, M.;[1] Milward, M.R.;[1] Cooper, P.R.[1]
[1]Oral Microbiology Group, School of Dentistry, University of Birmingham, Birmingham, UK
[2]Institute for Microbiology and Infection, University of Birmingham, Birmingham, UK

Background: The important oral anaerobe *Fusobacterium nucleatum* has previously been shown to be a key player in the development of a pathogenic multi-species biofilm associated with chronic gum disease – periodontitis. In order to regulate biofilm formation, bacteria release a variety of signalling molecules, one type being cyclic di-nucleotides (CDNs). This study aimed to understand CDN production in *F. nucleatum* biofilms and the importance of this signalling in pathogenic biofilm development.

Methods: Mono-species *F. nucleatum* biofilms were grown for 3, 5 and 7 days, multi-species biofilms for 7 days. Intracellular CDNs were extracted and analysed using liquid chromatography-tandem mass spectrometry. Biofilm biomass was quantified using crystal violet. Biofilm architecture was visualised by Scanning Electron Microscopy (SEM) and changes in gene-expression were measured by qRT-PCR.

Results: *In vitro* biofilm quantification of five different *F. nucleatum* subspecies showed significant variability between them. Multi-species biofilms formed stable biofilms with complex structures (SEM). Furthermore, genes required for synthesis and hydrolysis of CDNs were shown to be differentially expressed over time. Interestingly, the level of intracellular CDNs within the biofilms was either low or undetected.

Conclusion: This study showed considerable differences between *F. nucleatum* subspecies alongside a number of common characteristics. Difference in CDN production could reflect levels of pathogenicity of the different *F. nucleatum* subspecies. Understanding the role of CDNs in biofilm development may offer therapeutic targets to prevent pathogenic biofilm formation leading to development of periodontitis and associated systemic diseases.

Email: s.a.kuehne@bham.ac.uk

FUSOBACTERIUM NUCLEATUM IN BIOPSIED TISSUES FROM COLORECTAL CANCER PATIENTS IN KOREA

Lee, H.;*[1] Kim, M.;[1] Lee, S.-T.;[1] Choi, J.R.;[1] Kim, T.I.;[2] Lee, K.[1]
[1]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, South Korea
[2]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, South Korea

Background: Many studies have reported on the role of bacteria in the development of colorectal cancer (CRC). However, the role of individual bacteria and their relationship with epidemiological characteristics is still unclear. In this study, we aimed to determine the prevalence of CRC-associated bacteria using quantitative real-time PCR (qPCR) or metagenomic analysis, and statistical correlation of patient demographics and clinical characteristics including alcohol consumption with CRC-associated bacteria.

Methods: We determined the prevalence of five CRC-associated bacterial species in 38 CRC patients (39 samples) and 21 normal individuals using qPCR, and the relative abundance of bacterial taxa in the gut microbiome was assessed using metagenomic analysis. Epidemiological characteristics were investigated by reviewing the medical records and by interviewing the patients.

Results: *Fusobacterium nucleatum* was the only bacterium that was significantly ($P < 0.0001$) more prevalent in the cancer tissue (82.1%) than that in the normal tissue (0%) by qPCR. Metagenomic analysis showed a significant correlation between six operational taxonomic units (OTUs), including the genera *Fusobacterium, Peptostreptococcus, Collinsella, Prevotella, Parvimonas*, and *Gemella* in patients with CRC. An integrated analysis using metagenomic data and epidemiological characteristics showed that alcohol consumption was significantly correlated with the abundance of *Fusobacterium* OTU.

Conclusions: *F. nucleatum* was significantly prevalent in both early and late stage cancer tissue as measured by qPCR. The correlation of alcohol consumption with the abundance of *Fusobacterium* OTU in cancer tissue discovered using metagenomics analysis suggests a possible link between alcohol metabolism and changes in the gut microbiome, and subsequent tumorigenesis caused by *F. nucleatum*.

Email: hmlee.labmed@gmail.com

EVALUATION OF THE EFFECT OF RIFAXIMIN TREATMENT IN *FUSOBACTERIUM* ABUNDANCE AND FECAL DYSBIOSIS IN DOGS

Pilla, R.;*[1] Menozzi, A.;[2] Bertini, S.;[2] Ishii, P.E.;[1] Lidbury, J.A.;[1] Steiner, J.M.;[1] Suchodolski, J.S.[1]
[1]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX USA
[2]Department of Veterinary Sciences, University of Parma, Parma, Italy

Fusobacterium is the most abundant genus in canine fecal samples and, unlike in humans, it is considered beneficial for dogs. Decreased abundance of *Fusobacterium* is associated with gastrointestinal disease, and metronidazole and tylosin, the most commonly prescribed antimicrobials for dogs with diarrhea, cause further decreases in its abundance. Therefore, the objective of this study was to evaluate the impact of rifaximin treatment, previously found to be as effective as metronidazole in dogs with chronic enteritis, in *Fusobacterium* abundance and in fecal dysbiosis index, and compare it to metronidazole. Fecal samples were collected from 13 dogs with diarrhea, randomly assigned to be treated with rifaximin (RIF 25 mg/kg q12h, n=6) or metronidazole (MET 15 mg/kg q12 h, n=7) for 10 days, at days 0, 10, 25 and 40. Fecal samples were also collected from healthy controls (HC, n=19). DNA was extracted an qPCR was performed for *Fusobacterium*, and the fecal dysbiosis index (DI) was calculated. Statistics analysis was performed with Friedman's test, and Kruskall-Wallis followed by Dunn's multiple comparisons test. As expected, metronidazole administration caused a significant decrease in *Fusobacterium* abundance compared to baseline ($p=0.008$) and HC ($p=0.018$), and a significant increase in DI compared to baseline ($p=0.016$) and HC ($p<0.001$). Rifaximin, instead, did not affect *Fusobacterium* abundance compared to baseline ($p=0.956$) and HC ($p>0.999$). While DI was not significantly different from baseline ($p=0.740$), at day 10 DI in RIF was significantly higher than HC ($p=0.031$). However, the median DI value at day 10 was smaller in RIF (0.4) than MET (2.4), indicating that rifaximin induced less gut dysbiosis than metronidazole. Our results indicate that, unlike metronidazole, rifaximin did not affect *Fusobacterium* abundances, and only caused mild dysbiosis. Further studies are needed to evaluate rifaximin as an alternative to metronidazole in the treatment of diarrhea in dogs.

Email: rpilla@cvm.tamu.edu

CYTOKINE SECRETION UPON *FUSOBACTERIUM NUCLEATUM* BINDING AND INVASION ACCELERATES COLORECTAL CANCER CELL MIGRATION

Udayasuryan, B.;*[1] Casasanta, M.A.;[2] Yoo, C.C.;[2] Peng, H.;[1] Umaña, A.;[2] Sanders, B.E.;[2] Slade, D.J.;[2] Verbridge, S.S.[1]
[1]Virginia Tech, Department of Biomedical Engineering and Mechanics, Blacksburg, VA USA
[2]Virginia Tech, Department of Biochemistry, Blacksburg, VA USA

The molecular mechanisms by which *Fusobacterium nucleatum* influences the tumor microenvironment to accelerate and exacerbate colorectal cancer (CRC) are poorly understood. We found that *F. nucleatum* host cell binding and invasion into HCT116 CRC cells specifically elicits the secretion of potent cytokines IL8 and CXCL1 that have been well documented to be involved in immune cell recruitment, cell migration, and metastasis. Using an antibody capture array and ELISA tests, IL8 and CXCL1 were identified as the primary cytokines being secreted by HCT116 cells upon infection. To test that the secretion patterns were influenced by binding and invasion, we developed a *F. nucleatum* mutant (ΔFap2) that lacked the surface adhesin Fap2, known to bind host surface sugars, that showed lower bacterial invasion and concomitant decrease in cytokine secretion. Using Transwell assays to quantify migration, we show a significant increase in cell migration in the presence of 100ng/mL IL8 and CXCL1, and when using conditioned media from infected cells, compared to that from a non-infected control, this migration is impeded in the presence of anti-IL8 and anti-CXCL1 antibodies. Furthermore, we found that this response is non-specific in mouse neutrophils and macrophages, where infection elicited the secretion of CCL3, CXCL2, and TNFα. Interestingly, we found that the addition of galactose sugars inhibits host binding and attenuates cytokine section and cellar migration. These data indicate that *F. nucleatum* can modulate specific immune and cancer cell signaling via paracrine and autocrine factors. A clinical strategy to control the acceleration of CRC in patients could be the targeting of host-cell binding by *F. nucleatum* and arresting signaling pathways that may result in cell metastasis. Prospective studies include unravelling the patho-epigenetics of infection and engineering a biomimetic tumor spheroid platform to study the invasive capabilities of infected and non-infected tumor cells *in vitro*.

Email: barath@vt.edu

UTILIZATION OF DNA METHYLTRANSFERASES FOR ENHANCED BACTERIAL GENETICS IN *FUSOBACTERIUM NUCLEATUM*

Umaña, A.;* Sanders, B.E.; Wozniak, B.F..; Williams, K.J.; Slade, D.J.
Virginia Polytechnic Institute and State University, Department of Biochemistry, Blacksburg, VA USA

Fusobacterium are Gram-negative anaerobic bacteria that are known for their genetic recalcitrance. Herein, we present the first characterization of *Fusobacterium nucleatum* 23726 DNA methyltransferases that greatly enhance genetic manipulation by bypassing restriction modification systems. We biochemically characterized the DNA methyltransferases mFnuI and mFnuII and show that purified enzymes or constitutive expression in *E.coli* efficiently protects plasmids against host nucleases during plasmid transformation. In summary, we provide the first characterization of DNA methyltransferases in *Fusobacterium*, and have developed a suite of enzymes that enhance our previously developed molecular tools for the genetic modification of *F. nucleatum*, thereby accelerating our understanding of how this opportunistic oral pathogen contributes to diseases including periodontitis, preterm birth and colorectal cancer.

Email: arianaut@vt.edu

INVESTIGATION ON CLINICAL AND BACTERIOLOGICAL CHARACTERISTICS OF TWO MAJOR SUBSPECIES OF *FUSOBACTERIUM NECROPHORUM*

Yamagishi, Y.;* Nakamura, A.; Suematsu, H.; Asai, N.; Koizumi, Y.; Mikamo, H.
Clinical Infectious Diseases, Aichi Med University, Aichi, Japan

Objectives: *Fusobacterium necrophorum*, a gram-negative and rod-shaped anaerobe, is a major human and animal pathogen. *F. necrophorum* is divided into subsp. *necrophorum* and subsp. *funduliforme*; subsp. *necrophorum* is mostly associated with infections in animals and subsp. *funduliforme* is most common as a pathogen in human infections. The purpose of this study was to investigate prevalence of *F. necrophorum* subspecies and to compare the clinical and bacteriological characteristics.

Methods: Total 13 isolates of *F. necrophorum* detected by MALDI-TOF MS (VITEK-MS and MALDI Biotyper MBT smart) from human infections in Aichi Medical University Hospital were consecutively collected between January 2017 and June 2018.

Morphological examination for colony formation and Gram stain also performed by one researcher. Thirteen isolates were also re-identified and subtyped with nucleotide sequences of 16S rRNA and *gyr*B, searched for homology using BLAST.

Results: Among 13 strains, 11 were identified as *F. necrophorum* by genetic analysis; 5 were *F. necrophorum* subsp. *necrophorum* (Fnn) 6 were *F. necrophorum* subsp. *funduliforme* (Fnf). The remaining two strains were identified as *F. gonidiaformans*. Of phenotypes, hemagglutination test showed higher titers in Fnn, compared with Fnf. From clinical aspects, there were no differences for gender and age. The most common isolated sites was deep neck and tonsils abscess (63.5%). Fnn had a higher rate of single bacterial infections. APACHEII score, SIRS score and SOFA score tended to be higher for Fnn, compared with Fnf. In all cases, surgical procedures were performed.

Conclusion: About half of the *F. necrophorum* detected at our hospital were Fnf.

Identification accuracy of mass spectrometry in *F. necrophorum* was about 85%. In order to improve identification accuracy and distinguish subspecies, MALDI-TOF MS (VITEK-MS and MALDI Biotyper MBT smart) requires revised library. Also, our data suggested that Fnn might be more pathogenic than Fnf from clinical aspects.

Email : y.yamagishi@mac.com

A SUITE OF GENETIC TOOLS TO ELUCIDATE THE ROLE OF *FUSOBACTERIUM NUCLEATUM* IN COLORECTAL CANCER

Zepeda Rivera, M.A.;*[1] Bullman, S.;[2] Johnston, C.D.[1]
[1]Vaccines and Infectious Disease Division, Fred Hutchinson Cancer Research Center (FH), Seattle, WA USA
[2]Human Biology Division, FH, Seattle, WA USA

Unbiased genomic analyses have reproducibly revealed an enrichment of the invasive anaerobic bacterium *Fusobacterium nucleatum* in human colorectal cancer (CRC) tumors relative to non-cancerous colorectal tissues. Infection with *F. nucleatum* in animal and cellular models has supported a cancer-promoting role for *Fusobacterium*. Following an extensive characterization phase (i.e. what microbes are present), cancer microbiome studies are now moving towards studies that address causation (i.e. what are these microbes doing and how are they doing it). A significant limitation in this regard is that the majority of bacteria that can be grown in a laboratory, including CRC-associated *F. nucleatum*, cannot be genetically engineered. This is largely due to the presence of innate defense systems that detect and degrade exogenous DNA. Our goal is to design and apply systematic methodologies to overcome these innate bacterial defenses in order to genetically engineer *F. nucleatum* strains isolated from human CRC tumors and associated metastases. Using SyngenicDNA, a synthetic microbiology approach, we overcome the most widespread defense systems to create a suite of tailor-made genetic tools for a collection of *F. nucleatum* clinical isolates. We aim to apply these genetic tools to uncover novel bacterial factors required for *F. nucleatum* association with and invasion of human epithelial cells. These genetic tools will be vital when investigating the role of *F. nucleatum* in CRC onset, progression, and treatment.

Email: mzepedar@fredhutch.org

Gut Microbiome

Gut Microbiome

CLOSTRIDIUM BUTYRICUM AND *CLOSTRIDIUM NEONATALE* PATHOGENICITY AND NECROTIZING ENTEROCOLITIS

Ferraris, L.;[1] Balvay, A.;[2] Bellet, D.;[2] Delannoy, D.;[1] Klein, G.;[1] Maudet, C.;[2] Meilheuc, T.;[2] Butel, M.J.;[1] Rabot, S.;[2] Aires, J.;*[1] ClosNEC Study Group
[1]UMR-S1139, Faculté de Pharmacie de Paris, Université Paris, France
[2]Micalis, Inra, Université Paris Saclay, Jouy en Josas, France

Background: Necrotizing enterocolitis (NEC) is the most severe gastrointestinal disease of preterm neonates accounting for substantial morbidity and mortality in neonatal intensive care units. If NEC pathophysiology remains elusive, it is a multifactorial disease involving nutrition, immunological determinants and gut dysbiotic bacterial colonization. *Clostridium* sp. have been proposed as possible etiological agents of NEC.

Objective: Using a quail gnotobiotic anaimal model of NEC, we aimed at demonstrate the biological role of *Clostridium butyricum* and *Clostridium neonatale* in NEC pathogenesis.

Methods: *C. butyricum* and *C. neonatale* genetic knockouts in the *hbd* gene encoding a 3-hydroxybutyryl-CoA dehydrogenase (butanoate metabolism) were obtained (Clostron®). Germ free quails were inoculated with a culture of a wild type strain (WT group, n=13) or its *hbd*-deficient mutant (KO group, n=12). The animals' ceca were removed for macroscopic examination and scoring of the lesions. The cecal content was collected for bacterial enumeration. Histological analyses were performed on empty ceca.

Results: For both bacterial species the proportion of animals exhibiting NEC-like lesions was significantly higher in the WT than the KO group ($P=0.001$). The scores of macroscopic lesions were also significantly higher ($P<0.01$). Macroscopic lesions (haemorrhage foci, wall thickening) were confirmed by histological analyses for *C. butyricum* (inflammatory cells infiltration, ulcerations, wall thickening ($P=0.007$)), and to a lesser extent, for *C. neonatale* (inflammatory cells infiltration).

Conclusion: These experiments give new mechanistic data on NEC pathophysiology by unambiguously showing the importance of the butanoate metabolism of *C. butyricum* and, to a lesser extent, of *C. neonatale,* in NEC development.

Email: julio.aires@parisdescartes.fr

ENTEROTOXIGENIC *BACTEROIDES FRAGILIS* INDUCES TUMOR FORMATION VIA INCREASED MUTATION RATES AND CHROMOSOMAL INSTABILITY

Allen, J.;*[1] O'Hagan, H.;[2] Wu, X.;[1] Wu, S.;[1] Sears, C.L.[1,3]
[1]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD USA
[2]Medical Sciences Program and Cell, Molecular and Cancer Biology Program, Indiana University School of Medicine, Bloomington, IN USA
[3]Bloomberg-Kimmel Institute for Cancer Immunotherapy, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medicine Institutions, Baltimore, MD USA

Individuals born in 1990 have twice the risk of developing colon cancer as those born in 1950. The gut microbiome is being proposed as a potential contributor to this difference. Enterotoxigenic *Bacteroides fragilis* (ETBF) is one of the gut bacteria most studied in relation to colorectal cancer (CRC) development with the goal of determining if its elimination would decrease the risk of CRC. ETBF is found at higher frequency in CRC patients, and it rapidly induces tumor formation in an *Apc*$^{min/+}$ mouse model of CRC that is further exacerbated when *Msh2*, a gene functioning in the mismatch repair pathway (a key pathway in hereditary CRC), is knocked out. Importantly, *Msh2* knockout alone or combined with *Apc*$^{min/+}$ does not enhance colon tumorigenesis in the absence of ETBF. In this model, ETBF inoculation results in marked DNA damage in colon epithelial cells, but how this influences tumor development is unknown.

The purpose of this project was to understand how ETBF-induced genomic changes lead to tumor formation. To do this, we performed whole-exome sequencing on tumors and normal tissue isolated from *Apc*$^{min/+}$ and *Apc*$^{min/+}$ *Msh2*$^{-/-}$ mice after ETBF or sham inoculation. Our data indicate that ETBF-induced tumors contain more chromosomal breakpoints than sham tumors in both mouse strains, but more coding mutations only in *Apc*$^{min/+}$ *Msh2*$^{-/-}$ mice. Additionally, all tumor samples taken from *Apc*$^{min/+}$ mice show *Apc* loss of heterozygosity (LOH), while samples taken from *Apc*$^{min/+}$ *Msh2*$^{-/-}$ mice instead possess independent mutations in the *Apc* gene. These results indicate that in the presence of a functioning mismatch repair pathway, ETBF likely induces tumor formation by causing DNA damage that promotes chromosomal instability and increased rates of *Apc* LOH in colon epithelial cells. In the absence of a functioning mismatch repair pathway, such as in individuals with hereditary CRC syndromes (i.e. Lynch Syndrome), ETBF likely induces tumor formation by causing an increased mutation rate which eventually leads to a second inactivating hit in the *Apc* gene, a tumor suppressor gene. These data provide further evidence indicating that the elimination of ETBF from the gut microbiome may diminish the risk of CRC.

Email: jallen66@jhmi.edu

HYDROGEN GAS AS A REGULATOR OF FERMENTATION IN THE HUMAN GUT MICROBIOME

Campbell, A.C.;* Schmidt, T.M.
Department of Microbiology & Immunology, University of Michigan, Ann Arbor, MI USA

The influence of hydrogen gas (H_2) concentration on the metabolism of several common human gut microbes was assessed in order to investigate H_2 as a regulator of fermentation in the human gut microbiome. H_2 is a common product of carbohydrate fermentation by the microbiota of the human gut. Levels of colonic H_2 vary greatly from undetectable to over 40% of bowel gas, raising the possibility that H_2 concentration may be an important and underappreciated factor differentiating individual microbiomes, especially given its known influence on metabolic processes in anaerobic communities. We predicted that high concentrations of H_2 would increase production of the physiologically-relevant short chain fatty acid butyrate in the gut microbiome by thermodynamically inhibiting microbial hydrogenase. To test this hypothesis, cultures of several common human gut butyrate producers were grown under high and low partial pressures of H_2, as well as in the presence and absence of the hydrogenase inhibitor carbon monoxide (CO). Both increased butyrate production and decreased acetate production were observed under high H_2 and in the presence of CO. A notable exception to this finding was in *Faecalibacterium prausnitzii*, which lacks hydrogenase activity. Greatly increased lactate production was observed in *Eubacterium rectale*, an unexpected result nonetheless consistent with hydrogenase inhibition. We conclude that high colonic H_2 concentrations may effect physiologically relevant shifts in the profile of short chain fatty acids produced by the human gut microbiota, favoring butyrate production over acetate. Since butyrate is an important factor in colonic health associated with decreased inflammation and lower rates of colorectal cancer, this effect of H_2 on human gut microbial metabolism may play a role in human health. Going forward, we will explore whether a relationship exists between colonic H_2 concentration and short chain fatty acid production in human and mouse microbiomes *in vivo*. We will also investigate the effect of H_2 on the competitive fitness of different human gut bacteria in order to explore a potential role of H_2 in shaping the composition of the gut microbiome as well as its metabolic products.

Email: aacampbl@umich.edu

GUT BACTERIAL BILE ACID METABOLISM MODULATES COLONIC MOTILITY VIA EFFECTS ON THE ENTERIC NERVOUS SYSTEM

Li, N.;[1] Koester, S.T.;[1] Lachance, D.M.;[1,2] Dutta, M.;[3] Cui, J.;[3] Dey, N.*[1,4]
[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]Molecular Engineering & Sciences Institute, University of Washington, Seattle, WA USA
[3]Department of Environmental and Occupational Health Services, University of Washington, Seattle, WA USA
[4]Department of Medicine, Division of Gastroenterology, University of Washington, Seattle, WA USA

Gut motility is spatiotemporally dynamic, largely controlled by the enteric nervous system (ENS) and programmed to respond to the nutrient content of digesta, the gut microbiome, and microbiome-generated metabolites, with an overarching biological agenda of optimizing digestion and nutrition. Bile acids comprise a class of metabolites at the host-microbial interface, produced from cholesterol by the host and subsequently bio-transformed in myriad ways by gut bacteria, beginning with deconjugation by gut microbiome-encoded bile salt hydrolases (BSH). We previously showed that unconjugated bile acids generated by gut bacterial BSHs regulate gut motility in a diet- and ENS-dependent fashion. However, key ENS signaling pathways are unknown. To elucidate the extent to which gut bacterial bile acid metabolism regulate ENS signaling homeostasis and gut motility, we used a custom gene expression panel to profile wild-type and ENS-impaired *Ret*+/- gnotobiotic mice colonized with defined consortia of human gut bacterial strains varying in BSH activity and fed a diet containing or lacking turmeric, a cholekinetic dietary ingredient. Mice harboring gut microbes with high BSH activity had significantly faster whole gut transit, which we found to be robust to different gut microbiota and attributable to faster colonic motility. Turmeric alone induced expression of the ENS-specific gene *Ednrb*, which is critical in promoting ENS development. Gut microbial colonization correlated with significant changes in expression of *Glp2r*, which mediates growth signals to intestinal epithelial cells, though directionality was dependent upon specific microbiota and functional *Ret* signaling. These data suggest that ENS signaling homeostasis varies in a microbiota- and bile acid-dependent fashion.

Email: ndey@fredhutch.org

CORIOBACTERIIA ARE DIVERSE AND THIS IS REFLECTED IN MAMMALIAN GUT MICROBIOTAS

Hoyles, L.*
Nottingham Trent University, Nottingham, Nottinghamshire, UK

The class *Coriobacteriia* includes numerous fastidious anaerobes found within mammalian gut microbiotas. *Collinsella aerofaciens* is a core member of the Western human gut microbiota, while less abundant members of the class are involved in drug and bile acid metabolism (*Eggerthella*), equol production (*Asaccharobacter*, *Paraeggerthella*, *Slackia*, *Enterorhabdus*) and urolithin production (*Gordonibacter*, *Ellagibacter*) in humans and rodents. Recent 'culturomics' studies have greatly increased the number of *Coriobacteriia* recovered from human-associated samples, with numerous novel species (33) and genera (12) described. Incorporation of these newly described bacteria into taxonomic frameworks has been inconsistent, limiting the ways in which data from sequence-based studies involving humans and rodent models are interpreted. Consequently, the ecological range and genomic diversity of the class *Coriobacteriia* are poorly understood. The purpose of this study was to correctly annotate publicly available *Coriobacteriia* sequences, so that these data could be used to characterize *Coriobacteriia* in a range of different gut-derived datasets. 16S rRNA gene- and whole-genome-sequence-based analyses (average nucleotide identity, phylogenetics) were used to correctly assign bacteria to the families *Atopobiaceae*, *Coriobacteriaceae*, and *Eggerthellaceae*, and characterize their metabolic potential. Newly annotated 16S rRNA gene sequence data were searched against 85,000 datasets included in the Integrated Microbial Next Generation Sequencing database to determine the effect of using a manually curated database to study *Coriobacteriia* populations in a range of environmental samples. Members of the family *Coriobacteriaceae* predominated in the human gut, while *Eggerthellaceae* were more representative of the rodent gut. This was reflected in analyses of shotgun metagenomic data with a manually curated Kraken database. eggNOG-mapper analyses showed the metabolic capabilities of the three families of *Coriobacteriia* vary greatly, with *Eggerthellaceae* asaccharolytic compared with *Coriobacteriaceae* and *Atopobiaceae*. Current databases need to be updated to determine the contributions of the *Coriobacteriia* to different ecosystems.

Email: lesley.hoyles@ntu.ac.uk

BACTEROIDES THETAIOTAOMICRON AS A CHASSIS FOR UNDERSTANDING GUT-RESIDENT BACTERIOPHAGES

Hryckowian, A.J.;*[1,2,3] Porter, N.T.;[4] Merrill, B.D.;[3] Gardner, J.O.;[3] Singh, S.;[4] Nelson, E.J.;[5] Garlena, R.A.;[6] Russell, D.A.;[6] Martens, E.C.;[4] Sonnenburg, J.L.[3]
[1]Department of Medicine, University of Wisconsin Madison, Madison, WI USA
[2]Department of Medical Microbiology and Immunology, University of Wisconsin Madison, Madison, WI USA
[3]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA USA
[4]Department of Microbiology and Immunology, University of Michigan School of Medicine, Ann Arbor, MI USA
[5]Emerging Pathogens Institute, University of Florida, Gainesville, FL USA
[6]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA USA

Our emerging view of the gut microbiome largely ignores the roles and identities of bacteriophages (phages) in this ecosystem. Though phages are abundant in the gut, methods of data generation and analysis routinely used in microbiome science neglect phage biology, leaving important basic questions unaddressed. Which bacterium does a predicted phage infect? How are ecosystem dynamics impacted by phages? How do phages impact the health of the eukaryotic host? To help fill these gaps in understanding, we isolated a large collection of phages from the United States and Bangladesh that are specific to the prominent human gut symbiont, *Bacteroides thetaiotaomicron*. Using a panel of isogenic *B. thetaiotaomicron* mutants, we show that multiple phase variable mechanisms, including capsular polysaccharides (*cps*), modify bacteriophage susceptibility. Because the expression of *cps* by *Bacteroides* in the gut is affected by inputs such as inflammation and host diet, we are using these phages to test foundational hypotheses on the roles of phages in the response of the gut microbiome to ecological disturbances. Additionally, genomic analysis of these phages and comparison to existing metagenomic datasets reveals unexplored sequence space. Taken together, this work provides a foundation and direction for uncovering the identities and roles of phages in the gut microbiome, with relevance for how we treat the microbiome-host interactions central to our health.

Email: hryckowian@wisc.edu

GUT BACTERIAL COMMUNITIES FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION AND GRAFT VERSUS HOST DISEASE

Kowalsky, J.;*[1] DeMeules, M.M.;[1] Fiedler, T.L.;[1] Pergam, S.A.;[1,2] Wu, M.;[1] Hoffman, N.;[2] Quinn, M.;[1] Proll, S.C.;[1,2] Loeffelholz, T.;[1] Srinivasan, S.;[1] Fredricks, D.N.[1,2]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]University of Washington, Seattle, WA USA

Despite efforts to minimize graft versus host disease (GvHD) through HLA matching of donors and recipients, GvHD remains a major concern following hematopoietic cell transplantation (HCT). Previous studies have shown connections between the gut microbiome and GvHD. Here, we determined if changes in the gut bacterial alpha diversity is associated with GvHD.

Stool samples were collected pre-transplant and weekly for 100 days post-transplant in a cohort of patients undergoing allogeneic HCT (n=225). Clinical charts were reviewed for stage of gut GvHD. Next generation sequencing (NGS) of 16S rRNA gene amplicons was used to characterize bacterial communities within the gut. Alpha diversity was calculated using Shannon Diversity Index (SDI).

132 patients (58.7%) developed GvHD of the gut after transplant. Of those recipients, 74.2% developed stage 1 gut GvHD, 11.4% stage 2, 6.8% stage 3, and 7.6% stage 4. Recipients who did not develop GvHD had an initial reduction in alpha diversity of the gut bacterial community from 2.37 (SDI) immediately post-transplant to 2.13 after one week. This was followed by a quick recovery of alpha diversity to a value stabilizing near pre-transplant levels (2.35 by week 3 post-transplant). In contrast, among patients developing stage 3 and 4 severe gut GvHD, alpha diversity decreased sharply post-transplant from 2.30 (SDI) to 1.72 one week after transplant and failed to increase to pre-transplant levels. While there was no association between pre-transplant alpha diversity of the gut microbiota and the resulting GvHD stage, there was significantly lower diversity (2.718 vs 2.381 $p<0.0001$) at the point of engraftment between stage 0-1 and moderate to severe GvHD (stage 2-4).

Severe GvHD is associated with a failure in recovery of the bacterial alpha diversity in the gut following transplant and significantly lower alpha diversity at the point of engraftment.

Email: jrkowals@fredhutch.org

DEVELOPMENTAL IMPACT OF TRICLOSAN ON INTESTINAL MICROBIOME AND ITS CORRELATION TO FECAL IGA

Lahiani, M.H.;*[1] Gokulan, K.;[1] Sutherland, V.;[2] Cerniglia, C.E.;[1] Khare, S.[1]
[1]Division of Microbiology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR USA
[2]National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC USA

Triclosan (TCS), a commercially used antibacterial agent, has raised concerns about its impact on humans and the environment. TCS has been detected in human urine, breast milk, and fecal samples; however, little is known about its impact on the intestinal microbiome and host mucosal immunity. In this study, we assessed how TCS can affect the intestinal microbiome of rats upon exposure during select stages of development [Gestational (GD), postnatal (PND) and Post-partum (PP)]. Adult female rats were gavaged with triclosan at 100, 500, and 1000 mg/kg body weight (bw) per day from GD6 to PP28. The new born pups were exposed to TCS via lactation for 12 days and then (from PND12 onwards) gavaged with 100 and 500 mg/kg bw per day. Fecal materials collected from dams and pups were subject to total viable count of culturable aerobic and anaerobic bacteria. The same samples were also analyzed by 16S rRNA sequencing. Further, the IgA bound to bacteria, as free fecal IgA, was quantified using ELISA. Moreover, fecal bound or unabsorbed TCS in fecal samples were quantified using HPLC. Results showed that the total viable anaerobic bacteria were decreased significantly by 59%, at GD18 after exposure to TCS (100 mg/kg bw), but were recovered by PP28. However, pups gavaged with the same dose showed significant 50% decrease of anaerobic bacteria. TCS-exposed rats have shown a significant decrease in the abundance of *Bacteroidetes* and *Firmicutes* with the increase of *Proteobacteria* at all three doses. The 16s rRNA sequencing showed that the total OTUs detected in fecal samples was in correlation with the levels of unbound IgA and the levels of fecal bound TCS. These results show that TCS may modulate intestinal microbiota of female adult rats and pups and activate mucosal immunity. Studies are ongoing to elucidate the impact of early TCS exposure on gut-mucosa associated immune responses.

Email: mohamed.lahiani@fda.hhs.gov

GENE-LEVEL METAGENOMIC ANALYSIS OF MICROBIOME SURVEYS IDENTIFIES NOVEL GUT ANAEROBES ASSOCIATED WITH COLORECTAL CANCER

Minot, S.S.;*[1] Golob, J.L.;[2] Willis, A.D.;[3] Dey, N.[4]
[1]Microbiome Research Initiative, Fred Hutch Cancer Research Center, Seattle, WA USA
[2]Michigan Medicine Infectious Disease, University of Michigan, Ann Arbor, MI USA
[3]Department of Biostatistics, University of Washington, Seattle, WA USA
[4]Clinical Research Division, Fred Hutch Cancer Research Center, Seattle, WA USA

The human gut microbiome has been experimentally linked with important human diseases, such as colorectal cancer, as well as the therapeutic response to immunotherapy, but it has proved challenging to identify individual bacterial strains which can recapitulate this causal effect in a controlled experimental model. While prior approaches have relied on the set of bacterial genome sequences which are available for analysis, we feel that this is likely to be vastly underrepresented with the collection of anaerobic microbes which are essential for proper gut microbiome physiology. To address this analytical challenge, we have developed and implemented a novel experimental approach for microbiome analysis which relies on metagenomic *de novo* assembly to identify co-abundant gene groups (CAGs) which are associated with human phenotypes from microbiome survey datasets. We show that this *de novo* CAG approach for gene-level metagenomic analysis provides novel insights linking understudied anaerobes with human health in populations worldwide. In addition, we describe a reproducible analytical pipeline which can be used by any microbiome researcher to perform CAG-oriented analysis of metagenomic datasets, complete with interactive visualizations to support non-computational end-users. Having established this analytical approach, we describe future directions in which we have started to validate this approach with experimental administration of human gut anaerobes in a gnotobiotic mouse model of intestinal tumorigenesis.

Email: sminot@fredhutch.org

EFFECTS OF BACTEROIDES ON THE GUT INFLAMMATORY RESPONSE IN CYSTIC FIBROSIS

Price, C.E.;*[1] Antosca, K.M.;[1,2] Ruoff, K.L.;[1] Valls, R.A.;[1] Madan, J.C.;[2] O'Toole, G.A.[1]
[1]Geisel School of Medicine at Dartmouth, Hanover, NH USA
[2]Dartmouth-Hitchcock Medial Center, Lebanon, NH USA

The purpose of this work is to elucidate the mechanism of *Bacteroides* influence on health outcomes in Cystic Fibrosis.

The human gut microbiome is made up of diverse microorganisms that influence a broad range of host health outcomes, including normal immune development and function. Work from our group has demonstrated that there is a significant correlation between gut microbiome diversity in patients with cystic fibrosis and risk of airway exacerbation. In cystic fibrosis, reduced microbial diversity in the gut microbiome correlates with earlier onset of the first clinical exacerbation in infants less than 1 year of age. Studies from our lab have revealed that children less than 1 year of age with cystic fibrosis have significantly lower relative abundance of *Bacteroides* in the gut (stool) microbiome than children without cystic fibrosis. *Bacteroides* are primarily beneficial commensal microbes that promote normal immune development. We hypothesized that a lack of *Bacteroides* may alter immune signaling in cystic fibrosis patients, leading to higher systemic inflammation and higher rates of clinical exacerbation. We utilized an *in vitro* transwell co-culture system to determine whether the presence of *Bacteroides* can modulate secretion of IL-8, a pro-inflammatory cytokine, in CRISPR-modified CFTR-/CFTR- Caco2 human gut epithelial cells. We observed a significant decrease in IL-8 production in the presence of *Bacteroides*. Additionally, live bacteria are not required for the reduction of IL-8 secretion, as supernatant from *Bacteroides* can also reduce IL-8 secretion in this system. A screen of *Bacteroides*-secreted metabolites has revealed a subset of metabolites that inhibit IL-8 secretion. Future work will focus on characterizing the mechanism of action of *Bacteroides*-secreted product(s) on IL-8 secretion. This work demonstrates that *Bacteroides* species can modulate cytokine production by human gut epithelial cells and that the absence of *Bacteroides* may be key to health outcomes for cystic fibrosis patients.

Email: courtney.e.price.gr@dartmouth.edu

HOST-MICROBIOME CROSS TALK MEDIATED BY UNIQUE AMINO ACID CONJUGATIONS OF HOST BILE ACIDS

Quinn, R.A.;*[1] Guzior, D.;[1] Melnik, A.;[2] Vrbanac, A.;[3] Bodai, Z.;[4] Fu, T.;[5] Evans, R.;[5] Huttenhower, C.;[6,7] Mazmanian, S.;[8] Knight, R.;[3,9] Dorrestein, P.[2]
[1]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI USA
[2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA USA
[3]Department of Pediatrics, University of California San Diego, La Jolla, CA USA
[4]Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA USA
[5]Salk Institute for Biological Studies, La Jolla, CA USA
[6]Broad Institute of MIT and Harvard, Cambridge, MA USA
[7]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA USA
[8]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA USA
[9]Department of Computer Science and Engineering, University of California San Diego, La Jolla, CA USA

Through 170 years of bile acid chemistry research, comprising over 40,000 publications, our knowledge of mammalian bile acid conjugation was limited to the amino acids glycine and taurine. By screening all organs of germ free (GF) and colonized mice with LC-MS/MS based metabolomics, we have discovered unique bile acids made by the microbiome that are produced by conjugation of cholic acid core with alternative amino acids in the gut. These microbially conjugated bile acids (MCBAs) are produced by the gut anaerobe *Clostridium bolteae* in the upper GI tract of mice. *C. bolteae* produced bile acids with all essential amino acids conjugated except for those with basic residues. Mass spectrometry data mining through GNPS spectral searching identified these compounds in human samples, including infants, and particularly those with disease. MCBAs are elevated in individuals with ulcerative colitis and are detected in infants as early as 2-weeks of age. Furthermore, they strongly agonize the human FXR receptor, which is a global regulator of bile acid metabolism. Gavage of mice with MCBAs induced downstream FXR target gene expression, including the reduction of bile synthesis through the *Cyp7a1* gene. Unlike the host produced molecules glycocholic acid and taurocholic acid, these novel molecules could not be deconjugated by the microbiota, thus, their action on the FXR receptor may be constitutive. The discovery of these molecules and their effects on host biology opens up a new era in bile acid research. Future studies are needed to understand how MCBAs affects microbiome dysbiosis and gut disease.

Email: quinnrob@msu.edu

Anaerobes in the Genital Tract

Anaerobes in the Genital Tract

CHARACTERIZATION OF A NOVEL PREVOTELLACEAE FAMILY ORGANISM FROM THE FEMALE GENITAL TRACT

Austin, M.N.;*[1] Romero, R.;[2] Hillier, S.L.[1,3]
[1]Magee-Womens Research Institute, Pittsburgh, PA USA
[2]Perinatology Research Branch, NICHD/NIH/DHHS and Wayne State University, Detroit, MI USA and Bethesda, MD USA
[3]University of Pittsburgh, Pittsburgh, PA USA

Background: An obligately anaerobic rod not matching any known taxonomic group was recovered from the vaginas of pregnant women using enhanced cultivation techniques. The objective of this investigation was to characterize this previously non-cultivable Gram negative rod and describe its association with bacterial vaginosis.

Methods: A novel microorganism was isolated from quantitative vaginal swab samples from 23 of 987 samples from pregnant women. Vaginal smears were evaluated for bacterial vaginosis (BV) based on Nugent criteria. Two strains of this organism were subjected to biochemical testing for phenotypic characterization in duplicate and whole genomic DNA extraction for direct sequencing using broad range 16S rRNA gene primers.

Results: This organism was recovered from 17 (8.2%) of 205 women with BV vs 6 (0.08%) of 782 women without BV (p= =<.001, Chi square test). Testing revealed a non-pigmented, anaerobic, bile sensitive, vancomycin, colistin and kanamycin resistant, Gram negative rod that is asaccharolytic, catalase, indole and urease negative. API 20 A testing revealed weak gelatin hydrolysis and acidification of glycerol. API rapid ID 32 A and ZYM testing detected arginine, leucyl glycine, leucine, tyrosine, alanine, histidine, glutamyl glutamic acid arylamidases and alkaline, and acid phosphatases and naphthol-AS-BI-phosphate, respectively. A comparison of the novel organism within GenBank using the NCBI BLAST tool revealed *Prevotella aurantiaca* and *P. pallens* as the closest cultivable matches with 89% sequence similarity to both species.

Conclusion: Preliminary phenotypic and genotypic testing suggest this organism represents a novel genus and species within the family *Prevotellaceae*. Continued characterization will further clarify its phylogeny. Further research is required to elucidate its role in reproductive tract sequelae.

Email: maustin@mwri.magee.edu

A NOVEL *DIALISTER* SPECIES PREVALENT IN WOMEN WITH BACTERIAL VAGINOSIS (BV)

Beamer, M.A.;*[1] Stoner, K.A.;[1] Austin, M.A.;[1] Romero, R.;[2] Wiesenfeld, H.C.;[1] Hillier, S.L.[1,3]
[1] Magee-Womens Research Institute, Pittsburgh, PA USA
[2]Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI USA and Bethesda, MD USA
[3]University of Pittsburgh, Pittsburgh, PA USA

The *Dialister* genus has the second most abundant reads by deep sequencing from women with Nugent BV. We isolated an anaerobic Gram-negative coccus, designated F00547, having a 16S ribosomal gene sequence similarity of 94% to *Dialister microaerophilus* and >99% to 59 "uncultured" sequences and one isolate (accession KP192307) in the NCBI BLAST database. We assessed whether F00547 may be a novel species of *Dialister* and has an association with BV.

We cultured vaginal swabs collected during 978 visits from 457 pregnant women and 180 visits from 61 non-pregnant asymptomatic STI-free women and endometrial tissue collected from 193 non-pregnant women with pelvic inflammatory disease. Vaginal Gram stains were evaluated for BV by Nugent criteria. All samples were inoculated on Brucella agar with 5% sheep blood and incubated anaerobically ≥ 4 days at 37°C. Following cultivation, DNA was extracted for Sanger sequencing of the 16S ribosomal gene. Two isolates were phenotypically tested using API ZYM, Rapid ID 32A, and 20A. Resistance to antibiotics and bile was determined.

F00547 was detected by culture in 50 (10.9%) pregnant and 12 (19.7%) non-pregnant women, and from 48/243 (20%) visits where BV was diagnosed vs 23/915 (2.5%) visits for women without BV (P<0.001). F00547 was also isolated from 2/193 (1%) endometrial samples, both of which were obtained from women with BV. No carbohydrate substrates were metabolized. There were intermediate reactions suggesting leucine arylamidase and naphthol-AS-BI-phosphohydrolase activities and a strong valine arylamidase activity. Similarities to *D. microaerophilus* were arginine, phenylalanine, alanine, and histidine arylamidase activities, and differences for F00547 included glutamic acid decarboxylase and no arginine dihydrolase activities. Both F00547 isolates were resistant to vancomycin, but not to colistin, kanamycin, and bile.

F00547 may represent a new species of *Dialister*, but more characterization will be needed to determine its taxonomic status. Its prevalence suggests that it may contribute to the BV environment.

Email: mbeamer@mwri.magee.edu

ASSOCIATION BETWEEN VAGINAL BACTERIA AND HIV ACQUISITION RISK AMONG AFRICAN WOMEN PARTICIPATING IN THE VOICE STUDY

Srinivasan, S.;[1] Richardson, B.A.;[2] Wallis, J.M.;[1] Fiedler, T.L.;*[1] Hoffman, N.;[2] Proll, S.C.;[1] Chirenje, Z.;[3] Livant, E.;[4] Fredricks, D.N.;[1] Hillier, S.L.;[4] Marrazzo, J.[5]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]University of Washington, Seattle, WA USA
[3]University of Zimbabwe, Harare, Zimbabwe
[4]Magee-Womens Research Inst., Pittsburgh, PA USA
[5]University of Alabama, Birmingham, AL USA

We previously identified seven vaginal bacteria associated with increased HIV acquisition risk among African women using quantitative PCR (qPCR). We sought to extend the search for high-risk bacteria using a sequential PCR approach.

African women participating in a randomized placebo-controlled trial of daily oral vs. vaginal tenofovir-based pre-exposure prophylaxis for HIV (VOICE study) provided vaginal samples. Cases (150 women who acquired HIV) and controls (436 women who remained HIV uninfected) were matched by study arm and site. The microbiota was characterized by 16S rRNA gene PCR and sequencing to assess associations between relative abundances of bacteria and HIV risk; taxa were ranked in descending order by score statistic using logistic models run on each taxon until p-value=0.1. Taxa ≥5% were selected for measurement by qPCR. Relationship between bacterial quantities and HIV risk was analyzed using Generalized Estimating Equation models and adjusted for potential confounders.

Vaginal bacterial diversity among cases was higher than controls (p=0.004). Twelve bacterial taxa associated with HIV risk were identified that were not previously described. Six of 12 taxa were selected for qPCR measurements. Quantities of 5 of 6 taxa were significantly associated with increased risk for HIV acquisition. These include bacterial vaginosis-associated bacterium 2 (adjusted odds ratio (aOR)=1.57; 95% CI 0.97, 2.56), Candidate Division TM7 (aOR=2.04; 95% CI 1.14, 3.65), *Prevotella amnii* (aOR=1.53, 95% CI 0.95, 2.46), *Porphyromonas* Type 1 (aOR=2.04, 95% CI 1.27, 3.28), and *Peptinophilus lacrimalis* (aOR=1.55, 95% CI 0.98, 2.44). *Dialister micraerophilus* was not associated with HIV risk.

A sequential PCR approach facilitated identification of new bacteria associated with increased HIV acquisition risk. Interventions to decrease high-risk bacteria could be explored as one approach to reduce HIV risk in women.

Email: tfiedler@fredhutch.org

POLYMICROBIAL AND SINGLE-SPECIES INFECTIONS WITH VAGINAL BACTERIA DISTINCTLY IMPACT METABOLIC MICROENVIRONMENT IN A 3-D HUMAN CERVICAL MODEL

Łaniewski, P.;* Herbst-Kralovetz, M.M.
University of Arizona, Phoenix, AZ USA

Bacterial vaginosis-associated bacteria (BVAB) have been linked to gynecologic and obstetric sequalae. However, there is a fundamental gap that exists in understanding the functional impact of these bacteria in the cervicovaginal microenvironment that may contribute to disease. Hence, our objective was to identify metabolic signatures associated with vaginal microbiota in the context of cervical epithelium. Three-dimensional (3-D) human cervical epithelial cell cultures were infected under anaerobic conditions with single-species or polymicrobial inocula. We tested a panel of BVAB species: *Gardnerella vaginalis*, *Prevotella bivia*, *Atopobium vaginae*, *Sneathia amnii*, *Sneathia sanguinegens*, and health-associated *Lactobacillus crispatus*. Cell culture supernatants were collected 24 h post infection for untargeted metabolomics analysis by liquid chromatography-mass spectroscopy. The analysis yielded 418 metabolites with known identity, which was similar to the number of metabolites detected in cervicovaginal lavage samples in our previous clinical study. Principal component and hierarchical clustering analyses revealed that *A. vaginae*, *Sneathia* spp., and a polymicrobial cocktail exert more similar profiles and clustered separately from *G. vaginalis*, *P. bivia*, *L. crispatus,* and uninfected controls. Random Forest analysis highlighted excellent predictive accuracy (93.75%) and identified biochemicals involved mostly in amino acid (e.g. hydroxyisocaproate) and nucleotide (e.g. cytosine) metabolism as top predictors of bacterial infections. Furthermore, we identified significant changes in energy metabolism with species-specific preferences of carbon source and catabolic pathways. *S. amnii* and the polymicrobial infections resulted in an increase in polyamines (e.g. spermidine). Dramatic changes in arginine pathway were observed with *S. amnii*, but also *A. vaginae* and polymicrobial infections, which might induce inflammation via nitric oxide. In conclusion, we demonstrated the utility of our 3-D model to recapitulate the cervicovaginal metabolic microenvironment and identified species-specific metabolic pathways, which may contribute to dysbiosis.

Email: laniewski@email.arizona.edu

FUNCTIONAL DIVERSITY AND METABOLIC POTENTIAL OF HUMAN VAGINAL BACTERIA AS REVEALED BY METAPROTEOMICS

Lee, E.M.;*[1,2] Srinivasan, S.;[1] Fiedler, T.L.;[1] Proll, S.C.;[1] Leiser, O.P.;[3] Purvine, S.O.;[3] Kaiser, B.L.D.;[3] Fredricks, D.N.[1,2]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]University of Washington, Seattle, WA USA
[3]Pacific Northwest National Laboratory, Richland, WA USA

Women with bacterial vaginosis (BV) are colonized by diverse bacteria. Vaginal bacterial peptides may offer insights into their functions and contribution to BV.

Metaproteomic profiles of vaginal fluid from 29 women (10 BV-, 19 BV+) were generated by mass spectrometry. Bacterial community composition was determined by 16S rRNA gene sequencing. Bacterial peptides were identified with MS-GF+ software with a false discovery rate of ~1%. We used a database representing all bacterial taxa present in at least one sample at >0.1% relative abundance with genomes available in NCBI (Dec 2019). Differential peptide expression was calculated by two-sided t-tests.

We identified 7,355 unique bacterial peptides. Using a cutoff of $p<0.01$, 207 of the peptides were differentially expressed between BV+ and BV- samples. Functional analysis showed that most differentially expressed peptides play a role in carbohydrate metabolism and transport. Since glycogen is a major source of carbon in the vagina, we performed a targeted search for glycogen phosphorylase and phosphoglucomutase, two key enzymes in glycogen catabolism; we identified 177 spectral hits that matched homologs of these proteins. 174 of these spectra matched proteins belonging to bacteria in the genus *Gardnerella*. We also analyzed proteins known to play a role in BV pathogenesis, including vaginolysin and lactate-dehydrogenase. We identified vaginolysin peptides from multiple species of *Gardnerella,* including unnamed species and lactate-dehydrogenase from lactobacilli and BV-associated bacteria. Many *Lactobacillus* lactate-dehydrogenase peptides mapped to regions of the enzyme that vary between different species.

We identified bacterial peptides that correlate with BV status and play a role in carbohydrate metabolism in the vagina. Our results suggest *Gardnerella* may be capable of directly utilizing glycogen. Additionally, the different homologs of lactate-dehydrogenase made by various lactobacilli may in part explain differences in their lactic acid production.

Email: emlee@fredhutch.org

CYTOTOXICITY AND INFLAMMATORY POTENTIAL OF THE BACTERIAL VAGINOSIS-ASSOCIATED BACTERIA *PEPTONIPHILUS, PORPHYROMONAS,* AND *EGGERTHELLA* IN 3-D HUMAN CERVICAL CELL MODEL

Maarsingh, J.M.;*[1] Herbst-Kralovetz, M.M.[1,2]
[1]Department of Obstetrics and Gynecology, College of Medicine-Phoenix, University of Arizona, Phoenix, AZ USA
[2]Department of Basic Medical Sciences, College of Medicine-Phoenix, University of Arizona, Phoenix, AZ USA

Bacterial vaginosis (BV) is the most common gynecological disorder and a predisposing risk factor for preterm birth, increased susceptibility to sexually transmitted infections, and pelvic inflammatory disease. BV is a polymicrobial condition characterized by depletion of health-associated lactobacilli and overgrowth of diverse anaerobic bacteria, such as *Gardnerella, Prevotella,* and *Atopobium*. Other less-prevalent BV-associated bacteria (BVAB) are also linked to gynecologic sequelae, including *Eggerthella*, *Peptoniphilus,* and *Porphyromonas* are associated with persistent BV and endometrial cancer, respectively. The pathogenic mechanisms employed by these less-abundant taxa remain understudied. Using monolayer and 3-D human cervical epithelial cell models, we investigated the cytotoxic and inflammatory potential of three understudied BVAB: *Peptoniphilus lacrimalis*, *Porphyromonas uenonis*, and *Eggerthella* sp. *Eggerthella* sp. was cytotoxic to epithelial monolayers and reduced viability by 38% ($p < 0.05$) after 24h infection at MOI 10. Using scanning electron microscopy clusters of *Eggerthella* sp. and *P. uenonis* were shown to induce membrane blebs indicative of apoptosis and loss of cell membrane integrity in 3-D epithelial cells after 4h post-infection at MOI 10. *P. lacrimalis* significantly upregulated proinflammatory mediators including *CCL20* expression ($p < 0.007$), as measured by qPCR, in 3D cervical cells after 24h infection at MOI 10 and secretion was validated by immunoproteomic assays. Our results reveal that in our 3-D epithelial model, *Eggerthella* sp. and *P. uenonis* are cytotoxic and *P. lacrimalis* induces a pro-inflammatory response, without cytotoxicity. Our reductionist approach will advance in complexity as we continue to define potential pathogenic mechanisms employed by each species alone or in combination to enhance our understanding of bacterial-host interactions in the context of BV.

Email: jasonmaarsingh@email.arizona.edu

ANALYSIS OF VAGINAL MICROBIOTA OF PATIENTS IN THE INFERTILITY TREATMENT IN JAPAN

Matsumoto, A.;*[1,4] Yamagishi, Y.;[1,2] Takahashi, S.;[3] Kuroki, Y.;[4] Minemura, A.;[4] Oka, K.;[1,4] Takahashi, M.;[1,4] Mikamo, H.[1,2]
[1]Department of Clinical Infectious Diseases, Aichi Medical University, Aichi, Japan
[2]Department of Clinical Infectious Diseases, Aichi Medical University Graduate School of Medicine, Aichi, Japan
[3]Takahashi Ladies Clinic, Gifu, Japan
[4]Miyarisan Pharmaceutical Co., Ltd.; Tokyo R&D Center, Saitama, Japan

Background: Dysbiosis of the vaginal microbiota (VM) which is typically dominated by *Lactobacillus* species might be responsible for the bacterial vaginosis (BV). BV might lead low pregnancy rate, spontaneous abortion and preterm delivery in the pregnant women. Although recent reports suggested that dysbiosis of VM would be highly associated with infertility, the details are not clear. There are few reports that analyzed the VM of the infertility treatment patients for Japanese women. Therefore, in this study, we analyzed VM of Japanese women during infertility treatment to elucidate the association between infertility and VM.

Materials/methods: In February 2019, we collected the vaginal samples of 16 women during infertility treatment at Takahashi Ladies Clinic, Gifu, Japan. Nugent score was calculated using vaginal discharges. VM was analyzed by 16S rRNA gene sequenced metagenomic analysis using Miseq platform. Further, we investigated whether women were able to get pregnant using medical chart.

Results: VM were clearly clustered into 2 groups. Seven patients were classified in cluster 1 (C1) and 9 patients were classified in cluster 2 (C2). C1 was characterized with *Lactobacillus* spp. dominant but C2 was constituted by not only *Lactobacillus* spp. but also *Escherichia* spp., *Gardnerella* spp., and a few other taxa. The mean Nugent score of C1 was 1.7 whereas that of C2 was 4.1. The women who were able to get pregnant were 4 patients (57.1%) in C1, 3 patients (33.3%) in C2.

Conclusions: Our results showed that VM of Japanese women during infertility treatment were divided into 2 groups. Nugent score was correlated with composition of VM. Further, the women who had the VM which was dominated by *Lactobacillus* spp. tended to be higher in pregnancy rate than other women. Concurrently, we are investigating not only the pregnancy rate but also the delivery rate.

Email: a.matsumoto@miyarisan.com

CHARACTERIZATION OF BACTERIAL VAGINOSIS-ASSOCIATED BACTERIUM 2 (BVAB2) AND BVAB2-LIKE ORGANISMS

Stoner, K.A.;*[1] Beamer, M.A.;[1] Austin, M.N.;[1] Srinivasan, S.;[2] Fredricks, D.N.;[2] Strenk, S.M.;[2] Fiedler, T.L.;[2] Achilles, S.L.;[1,3] Wiesenfeld, H.C.;[1,2] Hillier, S.L.[1,3]
[1]Magee-Womens Research Institute, Pittsburgh PA USA
[2]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[3]University of Pittsburgh, Pittsburgh, PA USA

BVAB2 is a novel member of the Clostridiales order based on 16S rRNA gene sequencing and has been considered non-cultivable. Our objective was to further characterize BVAB2 and BVAB-2 like isolates recovered from the female genital tract.

BVAB2 and BVAB2-like bacteria were isolated from vaginal swabs (n=11) and endometrial biopsy samples (n=6) inoculated on Brucella agar with 5% sheep blood incubated anaerobically at 37°C for at least 4 days. BV was diagnosed from a Gram stained smear according to Nugent criteria. Four endometrial isolates were recovered from endometrial samples obtained from women with suspected pelvic inflammatory disease. All isolates formed very small colonies on Brucella agar. There was 96.5% sequencing identity between BVAB2 and BVAB2-like isolates based on sequencing of the 16S rRNA gene (1408 bp).

Women from whom BVAB2 was recovered (n=13) had a median Nugent score of 8 (range 5-10), and women from whom BVAB2-like was recovered (n=4) had a median Nugent score of 10 (range 3-10). Five isolates were selected for further phenotypic characterization: 2 endometrial and 2 vaginal of BVAB2 isolates and 1 endometrial BVAB2-like isolate. Both BVAB2 and BVAB2-like are small, non-motile, anaerobic Gram-positive coccobacilli that have slight β-hemolysis on Brucella agar. On comparison with validly named isolates, BVAB2 is most closely related to *Mageeibacillus indolicus* by 16S rRNA gene sequence identity (88.2%), formerly known as BVAB3. Phylogenetic analyses suggest that BVAB2 belongs to the family *Huntgateiclostridiaceae*. All isolates are colistin resistant, and vancomycin, kanamycin, and bile sensitive. Biochemical testing using API 20, API rapid 32 A, and ZYM revealed that these isolates utilize amino acids for growth.

BVAB2 and BVAB2-like bacteria may represent a new genus within *Huntgateiclostridiaceae.* Further characterization will be required to define the taxonomic status of these novel anaerobes.

Email: stonerka@upmc.edu

CHARACTERIZATION OF NOVEL *MEGASPHAERA* SPECIES FROM THE FEMALE REPRODUCTIVE TRACT

Srinivasan, S.;[1] Beamer, M.A.;[2] Fiedler, T.L.;[1] Austin, M.N.;[2] Sizova, M.V.;[3] Strenk, S.M.;*[1] Agnew, K.J.;[4] Nagana-Gowda, G.A.;[4] Rabe, L.K.;[2] Raftery, D.;[4] Epstein, S.S.;[3] Fredricks, D.N.;[1,4] Hillier, S.L.[2,5]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]Magee-Womens Research Institute, Pittsburgh, PA USA
[3]Northeastern University, Boston, MA USA
[4]University of Washington, Seattle, WA USA
[5]University of Pittsburgh, Pittsburgh, PA USA

Molecular studies have associated novel *Megasphaera* species with bacterial vaginosis (BV), preterm birth, and increased HIV acquisition risk. Here, we report the characterization of three novel phylotypes of *Megasphaera* cultivated from the female reproductive tract.

Three *Megasphaera* isolate types from the vagina and endometrium were identified by 16S rRNA gene sequencing and phylogenetic analyses. Two representatives of each isolate type were evaluated for phenotypic characteristics, carbon source utilization, cellular fatty acid composition, metabolic end products, and antibiotic susceptibility.

Two *Megasphaera* isolate types displayed 99.8% and 99.9% 16S rRNA gene sequence identity with *Megasphaera* sp. type 1 and 2, bacterial sequences previously detected in the vagina by 16S rRNA gene PCR and sequencing. *Megasphaera* sp. type 1, 2, and 3 had 16S rRNA gene sequence identity with *M. stantonii* (93%), *M. micronuciformis* (94%), and *M. cerevisiae* (95%), respectively, when compared with validly named species. All three types are strict anaerobic Gram-negative coccobacilli with cell sizes 0.5–1.5 μm. *In vitro* experiments and genome analyses revealed that the three isolate types metabolized amino acids but not sugars. Butyrate and butyric acid derivatives were noted as end product metabolites. There were specific differences between the three types; *Megasphaera* sp. type 2 and type 3 grew in broader temperature and pH ranges and only *Megasphaera* sp. type 3 metabolized alanine and glutamine. All isolates were susceptible to antibiotics typically used to treat BV, such as metronidazole and clindamycin.

Reproductive tract isolates of *Megasphaera* fall within the *Megasphaera* genus by phylogeny, but are sufficiently different to warrant designation as three new species. Availability of clinically important *Megasphaera* isolates provides an opportunity to explore how these bacteria affect the health of women.

Email: sstrenk@fredhutch.org

USE OF SHOTGUN METAGENOMICS TO INVESTIGATE THE PATHOGENESIS OF INCIDENT BACTERIAL VAGINOSIS

Taylor, C.M.;*[1] Toh, E.;[2] Dong, Q.;[3] Van Der Pol, W.J.;[4] Elnaggar, J.H.;[1] Luo, M.;[1] Lefkowitz, E.J.;[4] Nelson, D.E.;[2] Muzny, C.A.[4]
[1]Louisiana State University Health Sciences Center, New Orleans, LA USA
[2]Indiana University School of Medicine, Indianapolis, IN USA
[3]Loyola University, Chicago, IL USA
[4]University of Alabama at Birmingham, Birmingham, AL USA

Objective: We aimed to investigate changes in vaginal microbial community composition preceding incident bacterial vaginosis (iBV) using shotgun metagenomics.

Methods: Total DNA was extracted from stored vaginal swab specimens collected by women in a prospective BV pathogenesis study. Dual-indexed sequencing libraries were constructed using the NexteraXT DNA Library preparation kit (Illumina, San Diego, CA). Sequencing libraries were pooled at 12/lane and sequenced on 2 lanes of an Illumina HiSeq 4000, generating 150 base paired-end sequences, to an average depth of 7 gigabases. Sequences were processed using Kneaddata v0.7.2, MetaPhlAn2 v2.7.7, and Kraken2 v2.0.8 to determine taxonomic composition. Estimated read counts for each species were normalized across samples by total reads passing quality control.

Results: Sequencing was performed on vaginal specimens from 4 women who developed iBV (defined as a Nugent score of 7-10 on at least 2-3 consecutive days). Specimens collected every other day in the 10 days leading up to iBV were sequenced (6 specimens/woman). Across all 4 women, the percentage of microbial sequencing reads per sample increased substantially on the first day of iBV. Normalized estimated reads originating from the *Lactobacillus* genus declined while individual *Lactobacillus* species fluctuated differentially leading up to iBV. Normalized estimated reads of *Gardnerella vaginalis*, *Prevotella bivia*, and *Atopobium vaginae* increased sharply on the day of iBV.

Conclusion: The proportion of sequencing reads classified as microbial may provide an approximation of absolute bacterial abundance from total DNA isolated from vaginal swab specimens. The results of this pilot study suggest that the overall *Lactobacillus* abundance declines prior to iBV while the abundance of *Gardnerella vaginalis*, *Prevotella bivia*, and *Atopobium vaginae* increases sharply.

Email: ctay15@lsuhsc.edu

EFFECT OF METRONIDAZOLE ON VAGINAL BACTERIA ASSOCIATED WITH RISK OF HIV ACQUISITION

Valint, D.J.;*[1] Fiedler, T.L.;[1] Liu, C.;[1] Srinivasan, S.;[1] Fredricks, D.N.[1,2]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA USA
[2]University of Washington, Seattle, WA USA

Background: Women with bacterial vaginosis (BV) have complex vaginal bacterial communities. Several bacteria in these communities are associated with risk of HIV acquisition, but their susceptibility to antibiotics is poorly understood. We sought to characterize how vaginal concentrations of select bacterial taxa associated with increased HIV risk change following antibiotic treatment for BV in order to identify effective interventions for their eradication.

Methods: Vaginal samples were collected from 36 women enrolled in a longitudinal study of BV. Vaginal swabs were collected daily for two weeks following BV diagnosis, during which antibiotic treatment with metronidazole was administered either orally or as a vaginally applied gel for 5-7 days. DNA was extracted using QIAamp BiOstic Bacteremia DNA Kit and subjected to a suite of taxon-specific 16S rRNA gene quantitative PCR (qPCR) assays to monitor changes in concentrations of ten taxa associated with HIV infection risk. An anti-microbial test was developed to confirm the presence of antibiotics in vaginal swab samples using vaginal fluid inoculated onto plates with cultured bacteria.

Results: Bacterial DNA concentrations decreased over the duration of antibiotic administration for all bacterial taxa tested. Comparison of bacterial 16S rRNA gene copy numbers from samples taken before administration of antibiotics to samples taken on the last day of assay-confirmed antibiotic presence showed a 1.3-4.2 log-fold decrease across ten taxa [*Gemella asaccharolytica* (2.9), *Mycoplasma hominis* (1.8), *Parvimonas* type 1 (1.3), type 2 (3.8), *Megasphaera* type 1 (3.9), type 2 (2.9), Vaginal TM7 (4.2), *Prevotella amnii* (3.8), *Porphyromonas* type 1 (2.0), *Peptoniphilus lacrimalis* (2.2)]. In many instances, qPCR copy number was reduced to the assay's limit of detection, suggesting eradication of bacteria.

Conclusions: Administration of metronidazole reduces quantities of vaginal bacterial taxa associated with increased risk of HIV acquisition. Eradication of high-risk vaginal bacteria using metronidazole is one promising avenue to explore for reducing women's risk for HIV acquisition.

Email: dvalint@fredhutch.org

Oral Microbiome

PRELIMINARY STUDY OF THE QUALITY OF THE ORAL FLORA IN PARKINSON'S PATIENTS

Samot, J.;[1,2] Ella Nguema, B.;[2] Damon-Perriere, N.;[3] Guehl, D.;[3] Laurens, B.;[3] Tison, F.;[3] Lopez Cuina, M.;[3] Badet, C.*[1,2]
[1]Univ. Bordeaux, Unité de recherche Œnologie, EA 4577, USC 1366 INRAE, ISVV, Villenave d'Ornon, France
[2]Pôle Médecine et Chirurgie Bucco-Dentaire, CHU de Bordeaux, France
[3]IMN - UMR 5293 - CNRS/Université de Bordeaux, France

Parkinson disease is one of the most common neurodegenerative disorder worldwide causing a reduction in daily life activities, including the ability to keep an effective oral hygiene. The systematic review of the literature, despite low levels of evidence, shows that there is an influence of this disease on oral health.

We aimed to carry out an interventional, preliminary study that will rely on indicators of oral health status. The inclusion criteria were major patients, with Parkinson disease, able to give written informed consent, with more than 6 teeth distributed on maxilla and mandible.

The Outcome Measures were: Decayed Missing Filled Teeth index score, presence of cariogenic bacteria in saliva using the CRT Bacteria test, Community Periodontal Index of Treatment Needs, presence of periodontopathogen bacteria on two subgingival sites using RTPCR, salivary pH and flow measurements. These data were collected before hygiene advices were given to patients, when necessary.

Thirty-five patients aged from 39 to 81 were included. They showed deteriorated oral conditions.

Twenty patients (57%) showed high level of the potentially cariogenic species *Streptococcus mutans* and *Lactobacilli sp*.

Among the identified potentially periodontopathogenic anaerobe species with a rate above the pathogenicity threshold, *Prevotella intermedia* (87.8%) was the most frequently found followed by *Tannerella forsytia* (81.8%), *Treponema denticola* and *Campylobacter rectus* (69.7%), *Parvimonas micra* (60.6%), *Fusobacterium nucleatum* (48.50%), *Porphyromonas gingivalis* (45.5%). *Aggregatibacter actinomycetemcomitans* was present at a pathogenic threshold in only 8% of patients and *Eikenella corrodens* in only 6%.

This study is the first study conducted in France on the oral health of patients with Parkinson disease (ClinicalTrials.gov Identifier: NCT03827551). Most patients have high levels of potentially pathogenic bacteria and preventive measures must be put in place in these at-risk patients.

Email: marie-cecile.badet@u-bordeaux.fr

ISOLATION, PARASITIC-GROWTH AND HOST-RANGE OF ULTRA-SMALL BACTERIA TM7

Bor, B.;*[1,2] McLean, J.S.;[3] Dewhirst, F.E.;[1,2] He, X.;[1,2] Shi, W.[1]
[1]The Forsyth Institute, Cambridge, MA USA
[2]Harvard School of Dental Medicine, Boston, MA USA
[3]University of Washington, Seattle, WA USA

Saccharibacteria (TM7) is the only phylum with cultivated members in Candidate Phyla Radiation group (>73 phyla). In addition, Saccharibacteria has been associated with multiple inflammatory diseases such as periodontitis and vaginosis. However, its biophysiological details and role in diseases are still unclear. The first isolated Saccharibacteria strain, TM7x, surprisingly grew on a group of closely related bacteria, revealing a unique epibiont symbiosis that is extremely uncommon. An investigation of the interaction between the two partners using various biochemical, microscopy, and sequencing methods show that the bacterial host (host) has decreased cell-growth and completely inhibited cell-division when associated with Saccharibacteria. Intriguingly, Saccharibacteria can be virulent and lethal for its bacterial host. The host, however, can rapidly evolve to a state of reduced susceptibility, which results in a long-term parasitic relationship in which both species persist. Moreover, based on these findings, new isolation method was developed and used to cultivate many more Saccharibacteria species. All the isolated species required bacterial hosts for its epibiotic growth, and displayed restricted host range. Comparative genomic analysis revealed that the host selection is dependent on the genomic features such as presence/absence of a particular group of genes. These genes encompass multiple functional categories, from cell membrane/wall components to central metabolism. More broadly, the universal feature of Saccharibacteria as an epibiont and their shared small genome size suggest that Candidate Phyla Radiation organisms may predominantly prefer a symbiotic lifestyle.

Email: bbor@forsyth.org

BIOFUNCTIONALIZED ZINC PEROXIDE NANOPARTICLES INHIBIT PERI-IMPLANTITIS ASSOCIATED ANAEROBES

Fröber, K.;[1] Bergs, C.;[2] Pich, A.;[2,3,4] Conrads, G.*[1]
[1]Division of Oral Microbiology and Immunology, Department of Operative Dentistry, Periodontology and Preventive Dentistry, RWTH Aachen University, Aachen, Germany
[2]DWI-Leibniz Institute for Interactive Materials e.V., Aachen, Germany
[3]Institute of Technical and Macromolecular Chemistry, RWTH Aachen University, Aachen, Germany
[4]Aachen-Maastricht Institute for Biobased Materials, Maastricht University, Geleen, The Netherlands

For dental implant,s the accumulation of anaerobic bacteria is a main reason for peri-implant inflammation, which untreated can lead to implant loss. Oxygen releasing substances may act as antibacterial agents. In this study glucose-1-phosphate (Glc-1P) biofunctionalized zinc peroxide (ZnO_2) nanoparticles of four different synthesis ratio (1-10:1) and sizes (4-5 nm) were tested against the anaerobes *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, as well as against *Aggregatibacter actinomycetem-comitans*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Lactobacillus paracasei*, and the yeast *Candida albicans*. Nanoparticles stabilized with o-phosphorylethanolamine, bis[2-(methacryloyloxy)ethyl] phosphate, or dioctyl sulfosuccinate instead of glucose were used as controls. For every combination of test strain and nanoparticle both, the minimal inhibitory (MIC) and minimal microbicidal concentration (MBC or MFC) were determined under different pH conditions in microtiter plates. Furthermore, transmission electron (TEM) and fluorescence microscopy after live-dead-staining was performed on selected combinations of pathogen and nanoparticle in order to visualize the interactions. The ZnO_2/Glc-1P nanoparticles had an inhibitory effect on gram-negative anaerobes and on *A. actinomycetemcomitans* with a pH-dependent MIC ≥ 25 µg/ml and MBC ≥ 50 µg/ml, while the gram-positive species tested and *C. albicans* were not inhibited. In TEM images, attachment of nanoparticle-chains to the bacterial outer membrane and subsequent penetration was found together with an intracellular oxygen release. For nanoparticles with other stabilizers than glucose, an invasion was only seen in elongated, dividing cells, possibly because of the more porous cell wall in the parting layer.

Email: gconrads@ukaachen.de

ISOEUGENOL-MODIFIED NANOGEL-COATINGS FOR IMPLANT PROTECTION

Shi, Y.;[1] Bergs, C.;[2] Pich, A.;[2,3,4] Conrads, G.*[1]
[1]Division of Oral Microbiology and Immunology, Department of Operative Dentistry, Periodontology and Preventive Dentistry, RWTH Aachen University, Aachen, Germany
[2]DWI-Leibniz Institute for Interactive Materials e.V., Aachen, Germany
[3]Institute of Technical and Macromolecular Chemistry, RWTH Aachen University, Aachen, Germany
[4]Aachen-Maastricht Institute for Biobased Materials, Maastricht University, Geleen, The Netherlands

For dental implants, the accumulation of bacteria during osseointegration or life is a main reason for peri-implant inflammation, which untreated can lead to implant loss. (Iso)Eugenol extracted from cloves is known for its antiseptic & antibacterial properties and is well established as component of dental products. In the present work, we developed a new route to synthesize functional aqueous nanogels based on hydrophilic poly(ethylene glycol) (PEG) and decorated with a controlled amount of surface-drafted covalently bound isoeugenol.

To prove the antimicrobial properties of the designed nanogels, we selected the typical periodontal pathogens *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*, plus a panel of other oral commensals (*Actinomyces viscosus*, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus oralis*, *S. parasanguinis*), Isoeugenol macromonomers (MA) with spacer lengths of n = 6, 9 and 44 repeating units and different concentrations (0.1 - 20 mol%) were incorporated into nanogels (particle radius 60-200 nm) and the products labeled "Isoeug-PEG_n-MA" with n corresponding to the number of repeating units in the spacer.

Susceptibility to isoeugenol nanogels with spacer lengths of n = 9, but not of n = 6 (too short) or n = 44 (too long), was observed for all Gram-positive bacteria (*A. viscosus*, *E. faecalis*, *S. aureus*, *S. oralis*, and *S. parasanguinis*) tested with minimal bactericidal concentrations (MBCs) between 31.25 µg/mL and 1000 µg/mL. The strictly anaerobic, Gram-negative species *P. gingivalis*, *P. intermedia*, and *F. nucleatum* were also susceptible to Isoeug-PEG_9-MA 1-20 mol% with MBCs between 125 µg/mL and 1000 µg/mL. Gram-negative aerobic species such as *E. coli* and *A. actinomycetemcomitans*, as well as the yeast *C. albicans*, were found to be susceptible to ≥ 1000 µg/mL Isoeug-PEG_9-MA 10 mol% only.

In summary, we have developed a methodology for the design of bioactive coatings based on functional aqueous PEG-nanogels. We demonstrated that isoeugenol-modified nanogels exhibit superior antibacterial properties against oral bacteria in general and obligate anaerobic key peri-implantitis pathogens in particular.

Email: gconrads@ukaachen.de

HUMAN ORAL *CAPNOCYTOPHAGA* SPECIES AND BETA-LACTAMASE PRODUCTION IN PREGNANT AND POST-PARTUM WOMEN

Gürsoy, M.;*[1] Algahawi, A.;[1] Härkönen, V.;[1] Harju, I.;[2] Könönen, E.[1]
[1]Department of Periodontology, Institute of Dentistry, University of Turku, Turku, Finland
[2]Clinical Microbiology Laboratory, Turku University Hospital, Turku, Finland

Objective: Human *Capnocytophaga* species play a significant role in health and disease as well as in opportunistic infections. Additionally, this microorganism may serve as an important contributor to the β-lactam resistance gene reservoir in the oral cavity. Here, we aimed to identify human oral *Capnocytophaga* species isolated from subgingival plaque samples and to determine their β-lactamase production throughout gestation and post-partum.

Methods: Seven reference strains and 403 clinical *Capnocytophaga* isolates—originating from 30 pregnant, non-smoking, systemically healthy, periodontitis-free women—were examined by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). In addition, all clinical isolates were tested for their β-lactamase production by chromogenic Nitrocefin Disk method (Sigma-Aldrich, Munich, Germany).

Results: The majority (74.7%) of the clinical isolates were identified to species level (ID score ≥2.00); *C. ochracea* (266/321, 82.9%), *C. sputigena* (25/55, 45.5%), *C. haemolytica* (6/23, 26.1%), and *C. granulosa* (4/4, 100%). Two reference strains, *C. leadbetteri* and *Capnocytophaga* genospecies AHN8471, were identified correctly only to genus level, since both strains are missing from the current MALDI BioTyper database. *C. ochracea* was detected in all subgingival plaque samples. Throughout pregnancy and/or post-partum, half of the women harboured ≥1 β-lactamase positive isolate. Overall, the minority (25/403; 6.2%) of the tested clinical isolates produced β-lactamase. Indeed, the majority (95%) of *C. ochracea* isolates and all four (100%) *C. granulosa* isolates were β-lactamase negative, whereas 6/23 (26.1%) *C. sputigena* isolates produced β-lactamase.

Conclusion: Out of human *Capnocytophaga* species, *C. ochracea* is a common finding in subgingival microbiota of pregnant, periodontitis-free women. Moreover, β-lactamase production vary between the *Capnocytophaga* species, but the common prevalence seems to remain quite steady throughout gestation and post-partum.

Email: mervi.gursoy@utu.fi

EXPLOITATION OF HOST BACTERIUM-ENCODED FUNCTION BY HUMAN ORAL LYTIC PHAGE TO FACILITATE INFECTION

Cen, L.;[1] Bedree, J.;[1] McLean, J.S.;[2] Le, S.;[3] He, X.*[1,4]
[1]Department of Microbiology, The Forsyth Institute, Cambridge MA USA
[2]Department of Periodontics, University of Washington, Seattle, WA USA
[3]Department of Microbiology, Army Medical University, China
[4]Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, MA USA

Bacteriophages (phages) are an integral part of human oral microbiome, and their role in modulating and shaping microbial communities have been implicated. However, the evidence for the presence of diverse and abundant phage population largely came from metagenomic analysis or the observation of virus-like particles within saliva/plaque samples, while the isolation of oral phage and investigation of their interaction with bacterial hosts are limited. Here, we report the isolation of LC001, the first oral lytic phage targeting *Actinomyces odontolyticus*. Lytic phage LC001 was isolated from saliva samples, and infected XH001, an *Actinomyces odontolyticus* strain previously isolated from human oral cavity. The genome of LC001 was sequenced using Miseq, and gene annotations were determined using Prokka. LC001-resistant XH001 mutants were obtained by plating the mixture of XH001 wildtype cells and LC001 on solid BHI agar and screening for resistant colonies. The phage adsorption assay was performed to determine if the mutants were defective in phage binding. LC001-resistant mutants were subjected to genome sequencing to identify the gene mutation. Identified XH001 gene was knockout via allelic replacement and its phenotype of LC001 binding and sensitivity were tested. qPCR was performed to detect the virion replication within host cells. Our data showed that oral lytic phage LC001 displayed remarkable strain specificity against XH001. Meanwhile, spontaneous nonsense mutations in XH001-encoded lytic transglycosylase (LT) led to LC001-resistant phenotype, while maintaining phage-binding capability. This was further confirmed by targeted mutagenesis of XH001-encoded LT gene, which resulted in the same phenotypes as observed for LT spontaneous mutation. Phages rely on virion-associated lysins, such as LT and/or endopeptidase, to break peptidoglycan layers of host bacteria for effective ejection of their viral genome into the cytoplasm to achieve successful infection. We demonstrated that a human oral phage LC001 can exploit host bacterium-encoded lytic transglycosylase function to facilitate virion genome entry.

Email: xhe@forsyth.org

PARVIMONAS MICRA: ISOLATION FROM DENTAL ABSCESSES AND DEVELOPMENT OF A TRACTABLE GENETIC SYSTEM

Higashi, D.L.;* McGuire, S.; Abdelrahman, Y.M.; Merritt, J.L.
Oregon Health and Science University, Portland, OR USA

Parvimonas micra is a Gram positive obligate anaerobe and a typical member of the human oral microbiome as well as various other microbiomes around the body. *P. micra* is strongly associated with the development of numerous oral and systemic polymicrobial infections as well as multiple types of cancer. Despite its association with numerous diseases, almost nothing is known about *P. micra* biology, largely due to its genetic intractability. In this study, we isolated a collection of *P. micra* strains directly from odontogenic abscess samples. We screened these isolates using a transformation protocol developed in our laboratory and found the majority of isolates to exhibit natural competence, with some isolates exhibiting surprisingly high rates of transformation. By exploiting its natural competence ability, we were able to employ cloning-independent methodologies to engineer a variety of targeted chromosomal genetic modifications directly within wild clinical isolates. As a tractable and highly efficient genetic system developed for *P. micra*, the methods presented here will serve as a valuable tool to facilitate genetic research of this largely understudied organism to help elucidate its role in human health and disease.

Email: higashid@ohsu.edu

OUTER MEMBRANE VESICLE FORMATION IN *PORPHYROMONAS GINGIVALIS* REQUIRES LIPID A C4′-PHOSPHATASE ACTIVITY

Alaei, S.R.;[2] Coats, S.R.;[1] Darveau, R.P.;[1] Jain, S.*[1]
[1]University of Washington, Seattle, WA USA
[2]University of Washington, Tacoma, WA USA

Removal of an acyl chain from the bacterial outer membrane molecule, lipid A, is a unique mechanism used by select pathogenic bacteria to evade the host's TLR4-mediated pro-inflammatory response. In *Porphyromonas gingivalis*, an anaerobic keystone periodontal pathogen, the initially synthesized penta-acylated bis-phosphorylated lipid A, a TLR4 agonist, is converted to tetra-acylated structures, rendering it TLR4 evasive. We recently identified PGN_1123 as the gene encoding the lipid A deacylase in *P. gingivalis* 33277, and showed that a ΔPGN_1123 mutant harbors solely penta-acylated, predominantly non-phosphorylated, lipid A. Previously, we had identified PGN_1713 and PGN_0524 as the genes encoding lipid A C1- and C4′-phosphatases respectively, rendering the lipid A of their isogenic mutants mono-phosphorylated.

It is becoming established that under-acylated lipid A contributes to the formation of outer membrane vesicles (OMVs), as was demonstrated recently in *Salmonella*. *P. gingivalis* is a prolific OMV producer, and its OMVs have been shown to contain significantly more under (tetra)-acylated lipid A than the outer membranes from which they are derived. Our aim was to test OMV production in *P. gingivalis* penta-acylated lipid A mutants, with the hypothesis that these mutants are abrogated for OMV formation. We compared 33277 with isogenic ΔPGN_1123 and ΔPGN_0524 mutants. We had previously shown that lipid A of the ΔPGN_0524 C4′-phosphatase mutant is penta-acylated, indicating that the C4′-phosphate group needs to be removed for deacylation to occur. Transmission electron microscopy of cell-free supernatants revealed that ΔPGN_1123 mutants, harboring penta-acyl non-phosphorylated lipid A, is not significantly abrogated for OMV production relative to wild-type. However, the ΔPGN_0524 mutant, also possessing penta-acyl lipid A but bearing a C4′-phosphate, is severely attenuated for OMV formation. Hence, removal of the lipid A C4′-phosphate is required for OMV formation, perhaps in concert with deacylase activity. We are investigating site-directed PGN_0524 point mutants to evaluate if residues required for C4′-phosphatase activity are also required for OMV formation.

Email: sumita@uw.edu

INCREASED VARIETY OF ACTINOMYCES INFECTIONS

Könönen, E.*
University of Turku, Turku, Finland

Actinomycosis, an indolent, slowly progressing granulomatous disease with typical hard mass-like lesion(s), and *Actinomyces israelii* (*Streptothrix israeli*), as its causative agent, were recognized for more than 100 years ago. Since then, numerous novel *Actinomyces* species have been described. Among those are *A. gerencseriae* and *A. graevenitzii*, which are the other *Actinomyces* spp. involved in actinomycosis besides *A. israelii*. Also unusual presentations of the disease have been reported; it may occur in a context of specific therapies like bisphosphonate-related osteonecrosis of the jaw, osteoradionecrosis, and anti-inflammatory drugs. Notably, a wide variety of infections other than actinomycosis can be connected to Actinomyces species in humans. In fact, only a minority of infections where *Actinomyces* spp. are found are classical actinomycoses. Current knowledge about specific disease associations includes especially *A. meyeri* in brain abscesses, *A. neuii* in soft tissue and foreign body infections, and *A. turicensis* in infections at the genitourinary tract. In these cases, *Actinomyces* sp. usually appears in polymicrobial consortia. Noteworthy is that patients with actinomycosis or other types of *Actinomyces*-related infections can be misdiagnosed initially on the basis of clinical and radiological findings. Therefore, suspecting *Actinomyces* and detecting the organism(s) in clinical microbiology laboratories for choosing a proper treatment modality is important in clinical settings.

Email: eijkon@utu.fi

MODIFIED SHI-MEDIA SUPPORT GROWTH OF DIFFICULT TO CULTURE BACTERIA

Lamont, E.I.;*[1] Gadkari, A.;[1] To, T.;[1] He, X.;[2] Shi, W.;[2] McLean, J.S.[1]
[1]University of Washington, Seattle, WA USA
[2]Forsyth Institute, Cambridge, MA USA

Many anaerobic oral bacteria in subgingival plaque communities have unknown requirements for growth and are difficult to culture in-vitro. Of the Candidate Phyla Radiation, (a group of uncultivated phyla with reduced genomes) only the strain TM7x in the phylum Saccharibacteria has been successfully cultured in-vitro as an ultrasmall epibiont on its bacterial host. In this study, we modified the composition of SHI-media (known to support TM7x growth) to create conditions for the growth of members of the phylum Saccharibacteria along with other difficult-to-culture subgingival bacteria. A subgingival aggressive periodontitis plaque sample was inoculated in SHI-media and grown anaerobically for 48hrs with varying concentrations of sucrose (0, 0.1, 0.5%), fetal bovine serum (FBS) (0, 10, 30, 50%), and mucin (0.1, 2.5, 8.0g/L). A 16S rRNA sequencing run was conducted on the resulting polymicrobial biofilms for each condition. Interestingly, our results revealed growth of a G3 member, an as of yet uncultivated group within the Saccharibacteria phylum. G3 grew in SHI-media containing 10% FBS, and SHI-media without sucrose. Our data support that serum (a major component of gingival crevicular fluid) supports growth of subgingival anaerobes, as well as G3 members along with their potential hosts. Likewise, an absence of sucrose in the media appears to limit growth of acidogenic bacteria and may better simulate in-vivo subgingival plaque conditions. However, we found that organisms like *Treponema spp.* and *Tannerella forsythia* (otherwise hard to grow in-vitro) were found in the polymicrobial communities grown in SHI-media with 10% FBS as well as SHI-media with 0.5% sucrose. The presence of certain acidogenic bacteria in higher sucrose content media may help the growth of these organisms. These results suggest altering the FBS and sucrose concentration in SHI-media may support growth of difficult-to-culture organisms from subgingival biofilms.

Email: elamont@uw.edu

ANAEROBIC CHROMOGENIC BACTERIA INVOLVED IN EXTRINSIC TOOTH COLORATIONS

Lopez Nguyen, D.;*[1] Nancy, J.;[2] Badet, C.;[1,3] Saint-Marc, M.;[3] Etournaud, C.[3]
[1]Pôle Médecine et Chirurgie Bucco-Dentaire, CHU de Bordeaux, France
[2]Maison de santé Protestante de Bordeaux Bagatelle, Bordeaux, France
[3]University of Bordeaux, 146 rue Léo Saignat, Bordeaux, France

Black stains are extrinsic tooth colorations described as a thin, darkly pigmented line, localized on the cervical enamel following the contour of the gingiva. They occur at any age, but there seems to be a peak in childhood. Chromogenic bacteria, are suspected to be the cause of these characteristic pigmented stains, but only few studies are available.

The objective of this study was to identify anaerobic pigment-forming bacteria present in black stain and correlate its occurrence with the presence of one potentially cariogenic species *Streptococcus mutans*.

A total of 14 subjects with the chief complaint of black stains were selected based on the inclusion and exclusion criteria. 10 control patients were also recruited. Decayed/missing/filled surfaces score, plaque index, black stain score, and microbial analysis were performed.

Microorganisms of salivary and black stains samples were detected by cultivation on selective agar (Mitis Salivarius Agar, Columbia agar with sheep blood, Wilkins and Chalgren agar) and scored. They were identified phenotypically (Api 20Strep and Api 20ANA) and genotypically (DNA extraction and PCR method with specific primers).

The t-test was used to compare the results between the patient and the control groups.

The results showed that the number of CFU is significantly more important in salivary samples of patients with black stains. Moreover, there is significant bacterial diversity, as the flora found in black stains is rich and complex. *Actinomyces naeslundii* and *Prevotella melaninogenica* species, which have been shown by different authors to be the source of these black stains have been highlighted in stains samples.

However, in our study, the most recovered bacteria species was *Streptococcus mitis*.

We have not found any major influence of these stains on the salivary flora or on caries, although most studies tend to prove the contrary. Further studies should be driven to investigate the role of these stains on the oral ecosystem.

Email: nguyen.darrene@gmail.com

IMPROVEMENT OF A MULTI-SPECIES BIOFILM SIMULATING PERI-IMPLANTITIS

Badet, C.;*[1] Le Gac, C.;[2] Feuillolay, C.;[2] Roques, C.[2,3,4]
[1]Univ. Bordeaux, Unité de recherche Œnologie, EA 4577, USC 1366 INRAE, ISVV, Villenave d'Ornon, France
[2]Fonderephar, Faculty of Pharmacy, Université de Toulouse, Université Paul Sabatier, Toulouse Cedex 9, France
[3]Laboratoire de Génie Chimique, UMR 5503, Faculty of Pharmacy, Université de Toulouse, Université Paul Sabatier, Toulouse Cedex 9, France
[4]CHU Toulouse, Hôpital Purpan, Service de Bactériologie-Hygiéne, Toulouse, France

The aim of the project was to build up and validate an *in vitro* multi-species biofilm involving the main species implicated in peri-implantitis according to a protocol adapted from Guggenheim et *al.* (2001), on titanous carriers with roughness surfaces (implant model).

Biofilms were established on titanium carriers according to the Zürich biofilm model (Guggenheim et al., 2001). After saliva coating, carriers were covered with 50% saliva-50% modified universal fluid medium (mFUM). Microplate wells were inoculated with mixed bacterial suspensions. Then microplates were incubated anaerobically at 36 ± 1°C. In an attempt to improve biofilm maturation, the medium was renewed after 24h and 48h of incubation. *S. oralis* CIP 102922T or *S. mutans* CIP 103220T or *S. gordonii* CIP 105258T and *A. naeslundii* CIP 103128T were tested as pioneer strains. Secondary colonizers were *P. gingivalis* CIP 103683, *P. intermedia* CIP 103607, *P. micra* CIP 105294T, *F. nucleatum* CIP 101130T and *A. actinomycetemcomitans* CIP 52106T.

After biofilm formation, the number of colony-forming units (CFUs) was determined for each group/species. Confocal observations were also performed.

Preliminary experiments were performed to select the colonizers and their inoculum level which led to avoid *S. mutans* CIP 103220T and *S. oralis* CIP 102922T and to consider *S. gordonii* CIP 105258T. In such conditions, different experiments were performed regarding the medium (sucrose), the inoculum of the different strains and re-inoculation or not.

Conditions able to promote a multi-species biofilm with detection of all the strains were defined: Inoculum: *S. gordonii* 1^E3/ml, *A. naeslundii* 1^E5/ml, *P. micra* 1^E6/ml and Gram- 1^E7/ml (4 strains, each day); medium renewing each 24h on 72h. These conditions led us to demonstrate the dramatic loss of bactericidal activity of chlorhexidine digluconate and PVI on the peri-implantitis model versus planktonic cells (100/1). A dose-range effect was observed with CHX leading to a 4-5 log reduction at 5% (V/V). In the same conditions, PVI induced only a 3 log reduction even at 10% (V/V).

Email: marie-cecile.badet@u-bordeaux.fr

Probiotics

IMPACT OF *LACTOCOCCUS LACTIS* (PROBIOTIC SINUS RINSE) ON GROWTH OF PATIENT-DERIVED STRAINS OF *PSEUDOMONAS AERUGINOSA*

Cho, D.Y.;*[1,2] Skinner, D.;[1] Koch, C.G.;[1] Swords, W.E.;[2,4] Hunter, R.C.;[3] Crossman, D.K.;[5] Crowley, M.R.;[5] Rowe, S.M.;[2] Woodworth, B.A.[1,2]
[1]Department of Otolaryngology, University of Alabama at Birmingham (UAB), Birmingham, AL USA
[2]Gregory Fleming James CF Research Center, UAB, Birmingham, AL USA
[3]Department of Microbiology & Immunology, University of Minnesota, Minneapolis, MN USA
[4]Departments of Medicine & Microbiology, UAB, Birmingham, AL USA
[5]Department of Genetics, UAB, Birmingham, AL USA
[6]Departments of Medicine, Pediatrics, Cell Developmental & Integrative Biology, UAB, Birmingham, AL USA

Introduction: The *Lactococcus* strain of bacteria has been introduced as a probiotic nasal rinse for alleged salubrious effects on the sinonasal bacterial microbiome. The purpose of this study is to assess the interaction between *L. lactis* and patient derived *Pseudomonas aeruginosa*, an opportunistic pathogen in recalcitrant chronic rhinosinusitis (CRS).

Methods: Commercially available probiotic suspension containing *L. lactis* W136 was grown in an anaerobic chamber and colonies were isolated. Colonies were co-cultured with patient-derived *P. aeruginosa* strains in the presence of porcine gastric mucin (mimicking human mucus) for 72-hours. *P. aeruginosa* cultures without *L. lactis* served as controls. Colony forming units (CFU) were compared.

Results: Six *P. aeruginosa* isolates collected from 5 CRS patients (3 isolates from cystic fibrosis, one mucoid strain) and laboratory strain PAO1 were co-cultured with *L. lacts*. There was no statistical difference in CFUs of 5 *P. aeruginosa* isolates grown with *L. lactis* compared to CFUs without presence of *L. lactis*. CFU counts were much higher when the mucoid strain was co-cultured with *L. lactis* ($CFU_{+L.lactis}$=1.9 x10^8 ±1.44x10^7, $CFU_{-L.lactis}$=1.3x10^8±8.9x10^6, p=0.01, n=7). *L. lactis* suppressed the growth of one *P. aeruginosa* strain ($CFU_{+L.lactis}$=2.15x10^8±2.9x10^7, $CFU_{-L.lactis}$=3.95x10^8±4.8x10^6, p=0.03, n=7).

Conclusions: *L. lactis* co-cultured with *P. aeruginosa* in the presence of mucin induced growth in 1 strain, inhibited growth in another, and had no observable impact on 5 other isolates. Topical probiotic sinus rinse may not be universally provided as a 'one-size-fits-all' supplement and underlying mechanism needs to be explored.

Email: dycho@uabmc.edu

EFFECTS OF PROBIOTIC FERMENTATION ON THE ENHANCEMENT OF TYROSINASE INHIBITORY AND ANTIOXIDANT ACTIVITIES OF *CHENOPODIUM FORMOSANUM* LEAF EXTRACTS

Chen, P.Y.; Lin, J.L.; Cheng, C.Y.; Peng, C.C.; Chung, Y.C.*
Department of Biological Science and Technology, China University of Science and Technology, Taipei, Taiwan

Chenopodium formosanum (known as Red Djulis) is a native plant of Taiwan and its seed is a major source of colorants and antioxidants. *C. formosanum* leaves (CFL) are a type of agricultural waste. To improve physiological characteristics of CFL, this study evaluated the tyrosinase inhibitory and antioxidant activities of CFL extracts fermented by these probiotic bacteria: *Bifidobacterium bifidum*, *Lactobacillus brevis*, and *Streptococcus thermophiles* under anaerobic conditions. The CFL was first extracted using 70% ethanol and further fermented by probiotic bacteria for 24 h. The physiological characteristics of unfermented extracts (UE) and fermented extracts (FE) were evaluated. Results showed *B. bifidum*-fermented extract exhibited the highest physiological characteristics among the extracts fermented by these probiotic bacteria. The total phenolic and flavonoid concentrations were 516 mg-GAE/g-dried extract and 175 mg-QE/g-dried extract in *B. bifidum*-fermented extract. The antioxidant content of FE was 5.64 times greater than that of UE at least. The UE contained 12 kinds of antioxidative compounds and *B. bifidum*-fermented extract contained 17 kinds of antioxidative compounds at least. The primary anti-oxidative compounds were gallic acid, ferulic acid, vanillic acid, chlorogenic acid, rutin, quercetin, kaempferol and chrysin, which together accounted for 92.6% of the antioxidative compounds in *B. bifidum*-fermented extract. The IC_{50} values for antityrosinase activity, DPPH removal, and reducing power for *B. bifidum*-fermented extract were 252, 45.3 and 0.42 mg/L, respectively. Furthermore, the physiological activities of FE were considerably higher than those (905, 126.4 and 15.6 mg/L) of UE. The *B. bifidum*-fermented extract at 625 mg/L showed considerable cellular antityrosinase activity (95.2%) with low melanin production (9.6%) in human epidermal melanocytes (HEMn) and was noncytotoxic to HEMn and CCD-966SK cells. Thus, ethanol-extracted CFL fermented by *B. bifidum* may be used for developing new health food or cosmetic ingredients.

Email: ycchung@cc.cust.edu.tw

GENOMIC CHARACTERISATION OF CANDIDATE PROBIOTIC LACTOBACILLUS SPP. ISOLATED FROM SOUTH AFRICAN WOMEN

Wentzel, N.;[1] Kullin, B.R.;*[1] Happel, A.-U.;[1] Froissart, R.;[2] Passmore, J.-A.S.[1,3]
[1]University of Cape Town, Cape Town, Western Cape, South Africa
[2]CNRS, University Montpellier, Montpellier, France
[3]National Health Laboratory Service, Cape Town, Western Cape, South Africa

The female genital tract (FGT) microbiota plays an important role in overall reproductive health. Whereas an FGT microbial community characterised by a low vaginal pH and relatively high abundances of *Lactobacillus* spp. is generally thought to be protective, a dysbiotic vaginal microbiota, such as that which develops during bacterial vaginosis (BV), has been linked to a range of negative health outcomes. While probiotics have the potential to reduce the incidence of BV, there remain few affordable products containing vaginally derived strains specifically targeted at vaginal health in South Africa. In the current study, the genomes of five phenotypically well-characterised candidate probiotic strains (*L. crispatus* (X2), *L. jensenii*, *L.mucosae*, *L. gasseri*) isolated from vaginal samples provided by South African women, were sequenced and analysed for the presence of previously described antimicrobial resistance determinants, mobile genetic elements, putative prophages and CRISPR/CAS systems and potential bacteriocins. Three of the five strains harboured genes encoding putative TetM-type ribosomal protection proteins, while two possessed potential beta-lactamase class A proteins. However, none of these appeared to reside on or near to mobile genetic elements. Putative intact prophages were present in the genomes of two of the strains. CRISPR-Cas loci (type I-C, type II-A and type III-A) were identified in the genomes of three of the strains, with spacers showing homology to various lactococcal and streptococcal phages. Predicted helveticin J type bacteriocins were encoded by three of the strains. Together these results can be used to inform the development of these strains as probiotic candidates.

Email: brian.kullin@uct.ac.za

EFFECTS OF ANAEROBIC FUNGAL CULTURES AS PREBIOTICS, PROBIOTICS AND SYNBIOTICS ON *IN VITRO* RUMINAL FERMENTATION WITH RESPECT TO METHANE EMISSIONS

Choi, Y.Y.;[1] Lee, S.J.;[2] Kim, H.S.;[1] Eom, J.S.;[1] Kim, E.T.;[3] Lee, S.S.*[1,2]
[1]Division of Applied Life Science (BK21Plus) and Institute of Agriculture & Life Science (IALS), Gyeongsang National University(GNU)
[2]Institute of Agriculture and Life Science & University-Centered Labs, GNU, Jinju, South Korea
[3]National Institute of Animal Science, Rural Development Administration, Cheonan, Korea

Cultures of the anaerobic fungus, *Piromyces* M014 (isolated from the rumen of the Korean native goat, KACC 44951) as a symbiotic (SYN), autoclaved SYN as a prebiotic (PRE) and filtered SYN as a probiotic (PRO) were evaluated their ability to influence VFA production, cumulative and methane gas production, cellulose degradation and polysaccharidase enzyme activities by mixed ruminal microorganisms *in vitro* for developing feed additives and/or antimethanogenesis compounds. The addition of fungal cultures as a type of SYN, PRE and PRO significantly increased total volatile fatty acid. The molar proportion of acetate was decreased and that of propionate was increased, with a corresponding decrease in the acetate:propionate ratio. The addition of SYN, PRE and PRO caused a marked increase in total gas production, especially showing the significant ($P<0.01$) increase by 37, 32 and 21% after fermentation for 48 h, respectively, compared to anaerobic fungal medium as a control (CON). However, methane and hydrogen production decreased by the addition of fungal cultures. The decrease in methane accumulation relative to the control was 32.4, 24.9 and 11.7% for the SYN, PRE and PRO treatments, respectively. The similar responses of the three treatments were seen not only in the cellulose degradation but also in the enzyme activities of CMCase and xylanase compared with the CON. There may be several mechanisms involved in the positive responses of the addition of rumen fungal cultures (SYN and PRO) and their end-products (PRE) on the rumen fermentation and ecosystem. This is the first study showing the potential application of supplement of anaerobic fungal cultures as a type of probiotics, prebiotics and synbiotics may be applied industrially as an alternate feed additive that improves the feed quality and/or novel compound for antimethanogenesis.

Email: lss@gnu.ac.kr

PROPHYLACTIC USE OF A PROBIOTIC (*LACTOBACILLUS ACIDOPHILUS* CL1285, *LACTOBACILLUS CASEI* LBC80R, *LACTOBACILLUS RHAMNOSUS* CLR2) LED TO FEWER *CLOSTRIDIOIDES DIFFICILE* INFECTIONS AT A QUEBEC HOSPITAL

Maziade, P.J.;*[1] Ship, N.;[2] Sniffen, J.C.;[3] Goldstein, E.J.C.[4]
[1]Centre Intégré de Santé et de Services Sociaux de Lanaudière, Joliette, QC Canada
[2]Bio-K Plus International, Inc., Laval, QC Canada
[3]Florida Hospital Orlando, Orlando, FL USA
[4]R.M. Alden Research Laboratory, Los Angeles, CA USA

After years of endemic *C. difficile* infections (CDI), a Quebec hospital introduced a protocol that adds a specific probiotic daily adjunctive to antibiotic treatment.

The Centre Hospitalier Régional de Lanaudière initiated an antimicrobial stewardship program (ASP) in October 2016 and continued infection prevention measures. In October 2017, all adult inpatients taking antibiotics, 2 or more days, were eligible to receive probiotic (Bio-K+® 50 Billion capsules; *Lactobacillus acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2) from the start of antibiotic treatment. The rate and incidence during the intervention was compared to the preceding year. The incidence with and without probiotic prophylaxis during the intervention was compared.

Approximately 70% of eligible inpatients received probiotic prophylaxis during the 18 month intervention. The hospital-wide rate of nosocomial CDI decreased significantly, 5.2 vs 8.6 cases per 10,000 patient-days (P=0.001). There was a gradual decline at first, 6 months, followed by a significant decrease, 12 months, 7.7 and 4.0 CDI cases per 10,000 patient days (P=0.1, 0.0002). The incidence of CDI among eligible patients decreased from 1.5% to 0.9%, (P<0.001). Patients who received probiotic prophylaxis were disproportionately exposed to multiple types of antibiotic per visit (P<0.001), a critical risk factor for CDI. Comparing patients exposed to the same number of antibiotics, the incidence of CDI was significantly lower with probiotic prophylaxis and 2 or more, -50%, 3 or more, -65%, and 4 or more antibiotics per visit, -85% (P=0.009, 0.008, 0.0004, respectively).

Even with 70% application of the probiotic protocol, there was a significant decrease in the hospital-wide rate of nosocomial CDI. Among the patients taking multiple antibiotics, there was a significantly lower incidence of CDI for those taking this probiotic as prophylaxis.

Email: pierre-jean.maziade@ssss.gouv.qc.ca

A PROBIOTIC COMPRISED OF *LACTOBACILLUS ACIDOPHILUS* CL1285, *LACTOBACILLUS CASEI* LBC80R, *LACTOBACILLUS RHAMNOSUS* CLR2 PREVENTS *CLOSTRIDIOIDES DIFFICILE* INFECTIONS EVEN WHEN PATIENTS TAKE *LACTOBACILLUS*-ACTING ANTIBIOTICS

Millette, M.;*[1] Frappier, M.;[1] Ship, N.;[1] Maziade, P.J.[2]
[1]Bio-K Plus International, Inc., Laval, QC Canada
[2]Centre Intégré de Santé et de Services Sociaux de Lanaudière, Joliette, QC Canada

Bacterial probiotics are prescribed adjunctive to antibiotic treatment to prevent nosocomial *C. difficile* infections (CDI), though the antibiotics could inactivate them. Etest® for the 3 *Lactobacillus* sp. in a clinically useful probiotic showed susceptibility of each strain to amoxicillin, ampicillin, cefuroxime, imipenem and trimethoprim.

All adult inpatients at the Centre Hospitalier Régional de Lanaudière, Québec, taking antibiotics for 2 or more days were eligible to receive prophylactic probiotic (Bio-K+® 50 Billion capsules; *Lactobacillus acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2) from the start of antibiotic treatment. The incidence of CDI with and without probiotic prophylaxis was compared during the intervention, 10-14-2017 and 03-31-2019, and to the preceding year, 10-14-2016 to 10-13-2017.

Approximately 70% of eligible inpatients received probiotic prophylaxis during the 18 month intervention. Though 46% of the probiotic users took at least 1 *Lactobacillus*-acting antibiotic, the incidence of CDI decreased from 1.5% to 0.9%, (P<0.001). The incidence of CDI was somewhat lower with probiotic prophylaxis, 0.8%, than without, 1.1% (P=0.1); a trend also seen when *Lactobacillus*-acting antibiotics were used, 0.9% vs 1.2% (P=0.6). Patients receiving probiotic were more likely to be taking multiple antibiotics (P<0.001), and, a *Lactobacillus*-acting antibiotic (P<0.001). When comparing patients exposed to the same number of antibiotics per visit, and, at least 1 *Lactobacillus*-acting antibiotic, the incidence of CDI was lower with 2 or more antibiotics, -24% (P=0.5), and significantly decreased with 3 or more antibiotics, -69% (P=0.01).

Prophylaxis with this probiotic was successful even though nearly half of the probiotic users took antibiotics the bacterial strains are sensitive to *in vitro*. Patients taking three or more antibiotics had a significantly lower incidence of CDI with the probiotic, even with *Lactobacillus*-acting antibiotics.

Email: mmillette@biokplus.com

IMPROVED VIABILITY OF *CLOSTRIDIUM BUTYRICUM* BY CO-CULTURING WITH *LACTOBACILLUS* SPP.

Shin, Y.;*[1] Shin, C.;[1] Cho, S.;[1,2] So, J.-S.[1]
[1]Department of Biological Engineering, Inha University, Incheon 22212, Korea
[2]MEDIOGEN, Co., Ltd, Jecheon, Korea

Butyrate, a short-chain fatty acid produced by the intestinal bacteria, has been known to be linked to host's brain health. As one of butyrate-producing bacteria in intestinal tract, it has been suggested that *Clostridium butyricum* could attenuate chronic stress-induced depressive-like behavior. This study aimed to select and characterize lactic acid bacteria, which could improve the growth and function of *C. butyricum,* thereby increase the butyrate in the gut. When the viability of *C. butyricum* was examined after co-cultivating with supernatant of 249 lactobacilli, 25 strains showed significant improvement. Twenty five lactobacilli were further characterized in terms of their acid/bile tolerance and auto-aggregation ability for potential probiotics. One strain (YJ5) out of 25 lactobacilli was selected and identified as *Lactobacillus senioris* by 16s rDNA sequencing. When co-cultured, YJ5 supernatant increased the viability of *C. butyricum* up to 3.8 fold compared to the control. YJ5 also showed acid tolerance (4.7 log CFU/ml at pH 3 for 2h) and bile tolerance (37% survivability at 0.3% bile salt). Auto-aggregation ability of YJ5 was 84.5% after 5h incubation. *L. senioris* YJ5 is expected to enhance butyrate production of *C. butyricum.*, which is under study.

Email: ilovesea_5@naver.com

Resistance

LESS COMMON GRAM-NEGATIVE ANAEROBES: ANTI-MICROBIAL RESISTANCE PROFILE AND THE PREVE-LANCE OF KNOWN ANAEROBIC RESISTANCE GENES

Boiten, K.E.;* Baas, W.H.; Buijs, P.E.; Rossen, J.W.A.; Veloo, A.C.M.
University of Groningen, University Medical Center Groningen, Department of Medical Microbiology and Infection Prevention, Groningen, the Netherlands

There is insufficient information regarding antimicrobial resistance (AMR) in less common gram-negative anaerobes, like *Bilophila* spp., *Dialister* spp., and *Fusobacterium* spp. Therefore, we assessed the anti-microbial resistance (AMR) profile of this group of bacteria, including the prevalence of known antimicrobial resistance genes (ARGs).

166 isolates were consecutively isolated from human clinical samples between 2015-2019 representing the genera: *Alistipes* (n=7), *Bilophila* (n=41), *Dialister* (n=11), *Fusobacterium* (n=103) and *Sutterella* (n=4). Isolates were identified using MALDI-TOF MS and the AMR profile was assessed using E-test. Production of beta-lactamases was detected using nitrocefinase discs. Presence of ARGs was determined using a PCR targeting *cfxA*, *ermF*, *nim*, and *tetQ*.

In this study, 35,5% (59/166) of all isolates was resistant to amoxicillin. However, only 6% (10/166) produced beta-lactamase. Interestingly, all *Sutterella* isolates were resistant to clindamycin (4/4) and 3 isolates were also resistant to metronidazole. The *tetQ* gene was detected in 2/166 isolates. Both strains were *Alistipes* spp. and sensitive to tetracycline. No *cfxA*, *ermF* or *nim* genes were detected in the complete set of isolates.

There is no correspondence between the AMR profile and the presence of known anaerobic ARGs. Results indicate that a not yet described beta-lactamase may be present. Also, it remained unclear what the underlying mechanism is for metronidazole resistance in certain isolates and whether *erm* genes not detected by the primers were present. Further studies on this subject are warranted.

Email: k.e.boiten@umcg.nl

METALLO-BETA-LACTAMASE-MEDIATED CARBAPENEM RESISTANCE PREDICTION IN *BACTEROIDES FRAGILIS* VIA MALDI-TOF MASS SPECTROMETRY BRUKER SUBTYPING MODULE

Eberly, A.R.;* Shannon, S.K.; Bommersbach, C.E.; Walchak, R.C.; Schuetz, A.N.
Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN USA

Bacteroides spp. demonstrate increasing carbapenem resistance worldwide. Rapid resistance detection guides appropriate clinical therapy, but turnaround time of anaerobic phenotypic susceptibility results is long and testing is not typically performed by clinical labs. Metallo-beta-lactamase (MBL)-mediated carbapenem resistance in *Bacteroides* is mediated by *cfiA* with an upstream insertion sequence (IS). Recently available in the US, the RUO MALDI-TOF MS Biotyper® *B. fragilis* Subtyping Module (Bruker) identifies *B. fragilis* sensu stricto and predicts presence of *cfiA*. We compared results of the module to phenotypic susceptibility in *B. fragilis*, 24 testing intermediate (I) or resistant (R) to at least one carbapenem and 11 carbapenem susceptible (S). Isolates had previously been identified as *B. fragilis* by Bruker MALDI-TOF MS RUO BDAL v8468 library. Susceptibility testing by agar dilution or Etest (bioMérieux) was performed and results were interpreted by Clinical and Laboratory Standards Institute M100 anaerobe guidelines. All isolates correctly identified as *B. fragilis* by the module (scores >2.00). 22/24 (92%) carbapenem-R or -I isolates were predicted to contain *cfiA* by the module. 9/11 (82%) carbapenem-S isolates were *cfiA* negative. Overall agreement was 86% between the module and phenotypic carbapenem testing. In summary, the module identified *cfiA* in most carbapenem-R or -I *B. fragilis* isolates. False negatives may be due to presence of mechanisms other than MBLs, such as efflux pumps. False positives may be due to failure of the module to assess for presence of the upstream IS. The Subtyping Module allows early resistance detection on isolated colonies, shortening time to resistance prediction by 24-48 hours. Further studies using PCR to confirm the presence of IS, assessments of additional resistance mechanisms, and use of the module for *Bacteroides*/*Parabacteroides* spp. other than *B. fragilis* are needed.

Email: eberly.allison@mayo.edu

ANTIBIOTIC SUSCEPTIBILITY OF ANAEROBES FROM EUROPE OVER 12 YEARS (2007-2018)

Hackel, M.;* Bailey-Person, M.; Sahm, D.
IHMA, Schaumburg, IL USA

Objective: Anaerobic infections tend to be polymicrobial and are often treated empirically with broad-spectrum therapies. Susceptibility to the most commonly used antimicrobials among anaerobic pathogens varies among genera and even the species within a genus. Difficulty in isolating primary anaerobic pathogens and limited susceptibility testing by microbiology laboratories often results in the use of empirical treatment. For this reason, it is important to provide surveillance data to guide providers in the most effective choices for anti-anaerobic therapy. In this study, we report the susceptibility data for anaerobic clinical isolates from European hospitals from 2007 through 2018 collected through a surveillance study.

Methods: 12,074 anaerobic pathogens (6,967 Gram negative, 5,107 Gram positive) were collected from 2007-2018 from 24 sites in 9 countries in Europe (Belgium, Czech Republic, France, Germany, Hungary, Italy, Spain, Sweden, and United Kingdom). Gram negative organisms included *Bacteroides fragilis* Group (BfG), and *Prevotella* spp. Gram positive organisms included anaerobic cocci and *Clostridium* spp. excluding *C. difficile*. Organism identification was confirmed at a central laboratory (IHMA, Schaumburg, IL, US) by MALDI-TOF mass spectrometry and MIC values were determined using agar dilution following CLSI guidelines. Percent susceptible (%S) was calculated using 2019 CLSI breakpoints for cefoxitin (FOX), clindamycin (CLI), meropenem (MEM), metronidazole (MTZ), penicillin (PEN) and piperacillin-tazobactam (TZP), and FDA breakpoints for tigecycline (TGC).

Results: Greater than 90% of BfG and *Prevotella* spp. isolates were susceptible (S) to MEM, MTZ, TZP and TGC in 2016-2018. There was no significant decrease in %S over 12 years from 2007-2009, except for TGC, for which the %S for BfG isolates decreased from 97.5% to 91.2%. The %S of BfG to FOX decreased from 86.0% to 80.7% over this period, whereas the %S to CLI decreased from 68.6% to 61.6% for BfG, and from 77.6% to 68.2% for *Prevotella* spp. %S of *Clostridium* spp. to all drugs was maintained over this time, except for PEN, for which the %S decrease from 80.1% to 77.6%. %S of anaerobic cocci to all drugs was maintained over this time, except for CLI and PEN, for which the %S decreased from 88.4% to 82.5%, and from 95.1 to 91.8, respectively.

Conclusions MEM, MTZ, TZP and TGC continue to show *in vitro* activity against all anaerobic organisms isolated from European hospitals, with >96% S. CLI, PEN, and FOX exhibited lower activity overall. As antimicrobial resistance increases, differences in susceptibilities between genera highlight the need for continued evaluation of antimicrobial susceptibilities of anaerobic organisms to aid in the selection of empiric therapy and monitor resistance trends.

Email: mhackel@ihmainc.com

REAL TIME MONITORING OF ANTIMICROBIAL RESISTANCE IN ANAEROBIC BACTERIA

Morris, T.;* Ede, T.; Copsey-Mawer, S.; Scotford, S.; Anderson, B.; Davies, C.; Perry, M.D.; Hughes, H.C.
UK Anaerobe Reference Unit (UKARU), Public Health Wales, Cardiff, UK

'ARUMIC' is our newly developed web-based platform enabling users to view cumulative resistance data on all anaerobic isolates referred to our reference centre.

The prevalence of antimicrobial resistance in anaerobic bacteria is increasing. The consequences of this can be significant and it is therefore crucial that we regularly monitor the development of resistance to raise awareness amongst clinical teams.

The UKARU offers a UK-wide service for the identification and antimicrobial susceptibility testing (AST) of anaerobic bacteria from clinical sites. Since 2016, this service has included weekly AST by agar dilution as recommended by the Clinical Laboratory Standards Institute (CLSI). A standard panel of antimicrobials comprised of ceftriaxone (CRO), co-amoxiclav (AUG), clindamycin (CM), meropenem (MRP), metronidazole (MTZ), piperacillin-tazobactam (TZP), penicillin (PEN), and vancomycin (VA) are included.

The minimum inhibitory concentration (MIC) data is recorded in our Laboratory Information System (LIMS). The data is then curated before being transferred to a microbiology data suppository (Datastore). An automated link then generates an MIC distribution that can be visualised via a Tableau dashboard (business intelligence and analytics software). The dashboard allows users to select any combination of organism and antimicrobial and is updated weekly as new MIC data is generated.

The ARUMIC platform allows us to generate real time MIC population distributions for isolates referred from throughout the UK and further afield. Although this data is not from an unbiased surveillance population it does enable continuously updated monitoring of the development of resistance and also assists in the review and development of epidemiological cut-offs (ECOFFs) and species specific breakpoints.

Email: trefor.morris@wales.nhs.uk

METRONIDAZOLE RESISTANCE IN ANAEROBIC INTRA-ABDOMINAL INFECTIONS: A GROWING MENACE?

Padmaja Ananth, S.;* Shashidhar, V.
Department of Microbiology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India

Background: Intra-abdominal infections (IAI) comprise of diverse clinical conditions, ranging from uncomplicated appendicitis to life-threatening situations like florid faecal peritonitis which constitute the primary diagnosis in 8% of hospitalizations. Gut microbes are common etiological agents in intra-abdominal infections and *Bacteroides fragilis* group are the most commonly recognized anaerobic pathogens. In this study, we aim to characterize anaerobic isolates from IAIs and analyse their antimicrobial susceptibility against metronidazole.

Methods: Present study was conducted between January-December 2017 in the Department of Microbiology. Specimens such as pus aspirates, soft tissue, and peritoneal fluid from diverse IAIs were included. Anaerobes were identified following standard techniques and MALDI-TOF (Vitek MS, bioMérieux, France) was used for species identification. β-lactamase production was detected using nitrocephin disks. E test method (bioMérieux) was used to determine the antimicrobial susceptibility of metronidazole. The results were interpreted following Clinical Laboratory Standards Institute (CLSI) guidelines

Results: A total of 58 specimens from IAIs were analysed, of which anaerobic growth was observed in 39.7% (23) of specimens. Intra-abdominal abscess was the common presenting complaint. Anaerobic Gram negative bacilli (71.4%) were the common isolates, of which *B. fragilis* were frequent anaerobe in 45.7% (16) followed by anaerobic Gram positive cocci (22.9%). Metronidazole resistance was observed among 3 isolates (13%) of which, two were Prevotella spp. (MIC 32 μg/mL & 256 μg/mL), and one *B. fragilis* (MIC 32 μg/mL). β-lactamase activity was detected in all *B. fragilis* group isolates.

Conclusion: Increasing trend in metronidazole resistance among anaerobic pathogens may jeopardize its role as empirical therapeutic choice in future, particularly in developing countries which lack the facilities of routine anaerobic susceptibility testing. It is time to look into metronidazole resistance carriage among intestinal anaerobic flora and their probable horizontal transmission across species is the need of the hour

Email: padmajashenoy@hotmail.com

BETA-LACTAM RESISTANCE DEVELOPMENT AFFECTED BY THE INTERACTION OF PENICILLIN-BINDING PROTEINS (PBPS) OF *CLOSTRIDIUM PERFRINGENS* WITH THE FLUORESCENT PENICILLIN, BOCILLIN FL

Park, M.;* Sutherland, J.B.; Rafii, F.
National Center for Toxicological Research, Jefferson, AR USA

Alterations in the affinity and binding of bacterial penicillin-binding proteins (PBPs) to β-lactams are important in the development of drug resistance. The PBPs of wild type

Clostridium perfringens ATCC 13124 and three β-lactam-resistant mutants were compared for the ability to bind to a fluorescent penicillin, BOCILLIN FL. The binding of a high molecular weight protein, PBP1, a transpeptidase, to BOCILLIN FL was reduced in all of the resistant strains. In contrast, the binding of BOCILLIN FL to a low molecular weight protein, PBP6, a D-alanyl-D-alanine carboxypeptidase that was more abundant in all three resistant strains, was substantially increased. A competition assay with β-lactams reduced the binding of all of the PBPs, including PBP6, to BOCILLIN FL. β-Lactams enhanced transcription of the putative gene for PBP6 in both wild type and resistant strains. This is the first report showing that mutations in a high molecular weight PBP and overexpression of a low molecular weight PBP in resistant *C. perfringens* strains affected their binding to β-lactams.

Email: miseon.park@fdahhs.gov

ANTIMICROBIAL RESISTANCE OF *CLOSTRIDIUM* SPP. ISOLATES FROM SOFT TISSUE AND BONE INFECTIONS IN A COSTA RICAN TRAUMA HOSPITAL, 2017 – 2019

Sandí, C.;*[1] Quesada-Gómez, C.[2]
[1]Laboratorio Clínico Universidad de Costa Rica, Hospital del Trauma INS, San José, Costa Rica
[2]Laboratorio de Investigación en Bacteriología Anaerobia LIBA, Universidad de Costa Rica, San José, Costa Rica

Individuals suffering from deep open wounds and exposed fractures are at risk of acquiring infections by different species of *Clostridium*. The optimal treatment includes extensive surgical resection and an antimicrobial therapy. Twenty-four strains of *Clostridium* sp. of soft tissue and bone infections were isolated from hospitalized patients at the Trauma Hospital of the National Insurance Institute of Costa Rica. The majority of strains were isolated from tibia, knee, femur and elbow infections. The isolates were identified through MALDI-TOF and the following species were obtained: *Clostridium perfringens, Clostridium tertium, Clostridium sporogenes, Clostridium bifermentans,* and *Clostridium sordellii*. Afterwards, resistance percentages were obtained using ATB-ANA test: metronidazole 54%, imipenem 50%, clindamycin 38%, chloramphenicol 29%, cefotetan 21%, cefoxitin 17%, benzylpenicillin 12% and piperacillin 4%. All strains were sensitive to amoxicillin, amoxicillin/Clavulanate, piperacillin/tazobactam, ticarcillin, and ticarcillin/Clavulanate. *C. tertium* showed more resistance to at least tree antimicrobial families. The results will help the empirical management of these infections, which in some cases do not respond to first-line treatments.

Email: cindy.sandi@lcucr.com

INVESTIGATIONS INTO THE PHENOTYPIC CHARACTERISTICS AND MOLECULAR MECHANISMS OF THE HETEROGENEOUS CARBAPENEM RESISTANCE OF *BACTEROIDES FRAGILIS* STRAINS

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

The insertion sequence regulated, high-level carbapenem resistance of *B. fragilis* strains mediated by the *cfiA* gene is already well-known. However, besides this latter and the low-level ('silent') carbapenem resistance, strains are regularly found to show reduced susceptibility to imipenem (MIC 1-8 μg/ml) and gave heterogeneous resistance profiles with IP Etests. We aimed to characterize those resistance mechanisms because *B. fragilis* strains with this phenotype might not be treated effectively by carbapenems. It was also assumed that the GNAT and XAT acetyltransferase genes found in the vicinity of the *cfiA* genes are involved in this resistance mechanism as a toxin-antitoxin (TA) addiction pair.

Agar dilution and Etest MIC values were determined along with population profile analysis (PAP) experiments using 10 heterogeneously imipenem resistant *B. fragilis* strains. These latter values, imipenemase production and the expression data of the *cfiA*, GNAT and XAT genes measured by qRT-PCR were then correlated with each other.

Heterogeneously imipenem resistant strains tended to give broader growth ranges which were ≥3 in 2-fold dilution steps. They also produced higher specific imipenemase activities than silent strains. Imipenem MICs, imipenemase activities and gene expressions of the *cfiA*, GNAT and XAT genes well correlated ($r>0.8$).

We conclude that higher imipenemase production is responsible for imipenem heterogeneous resistance which somehow could be regulated by the GNAT-XAT TA pair deduced from the high correlation between the expression of *cfiA* and the GNAT toxin.

Email: soki.jozsef@med.u-szeged.hu

MOLECULAR CHARACTERIZATION OF METRONIDAZOLE-RESISTANT *BACTEROIDES* STRAINS FROM KUWAIT

Baaity, Z.;[1] Jamal, W.;[2] Rotimi, V.O.;[2] Burián, K.;[1] Nagy, E.;[1] Sóki, J.*[1]
[1]Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary
[2]Department of Microbiology, Faculty of Medicine, Kuwait University, Safat, Kuwait

Metronidazole resistance constitutes a significant issue among anaerobic pathogens, particularly in countries where antibiotics are not prudently used. Its best described resistance mechanism is mediated by *nim* gene-insertion sequence (IS) element pairs ,which can be questioned, but most of the metronidazole-resistant clinically important *Bacteroides* isolates do harbor them. Metronidazole resistance among *Bacteroides* strains is also a major concern along with the prominent rise of multidrug-resistant strains as well.

421 clinical *Bacteroides* strains were collected during 2006-2018 in Kuwait. Antibiotic susceptibilities were recorded by Etests and *nim* genes and IS elements were detected by PCR in metronidazole-resistant strains. *Nim* gene types were characterized by nucleotide sequencing and the localization of the *nim* genes was determined by plasmidic DNA isolation and Southern blotting. The genetic similarity of the metronidazole-resistant *Bacteroides* strains was investigated by Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) typing. Carbapenem resistance mechanisms were also analyzed using comparable methods.

Out of the 421 *Bacteroides* isolates 12 were proven to be metronidazole resistant (10 *B. fragilis*, *1 B. dorei* and 1 *B. thetaiotaomicron*). All but one was *nim* gene-positive harbouring the *nimE* gene. Of these, 9 were activated by IS*Bf6* and 5.7, 8.3, ~10 and ~13 kb plasmids harbored the *nimE* genes. Interestingly, 6 of the *nim*-positive strains were also *cfiA*-positive with 5 silent and 1 with phenotypic resistance. By means of ERIC-PCR typing, the *B. fragilis* strains were not clonal.

The prevalence of metronidazole resistant *Bacteroides* strains is around 4% in Kuwait, and it is mainly due to *nimE* gene and IS*Bf6*-carrying plasmids, which in our cases represented four molecular weight classes. Since most of the examined strains were also *cfiA*-positive, therefore possible treatment options are fairly limited.

Email: soki.jozsef@med.u-szeged.hu

METRONIDAZOLE SUSCEPTIBILITY OF CLINICALLY RELEVANT *BACTEROIDES FRAGILIS* GROUP BACTERIA IN A UNIVERSITY HOSPITAL IN ISTANBUL, TURKEY

Ulger Toprak, N.;*[1] Eminoglu, S.;[1] Tekes, B.;[1] Djerboua, T.;[2] Sayın, E.[3]
[1]Marmara University, School of Medicine Department of Microbiology, Istanbul, Turkey
[2]Nedir Mohammed University-Hospital, Belloua Unit, Clinical Biology Laboratory, Tizi-Ouzou-Algeria
[3]Marmara University Pendik Education and Research Hospital, Istanbul, Turkey

Metronidazole is often used as empirical therapy for *Bacteroides fragilis* group (BFG) bacterial infections. However, acquired resistance to metronidazole among BFG bacteria has been reported from different countries. This is worrisome, especially since such resistance has been described in multidrug-resistant BFG bacteria. The main metronidazole resistance mechanism has been associated with the *nim* genes.

In this study, we aimed to obtain susceptibility data for metronidazole against BFG bacteria isolated from human infections, we also wanted to investigate the presence of *nim* genes in these isolates. In total 211 BFG bacteria isolated from different clinical samples were tested. Organisms were identified by using MALDI-TOF MS. MICs of metronidazole was determined using agar dilution methodology (CLSI; M11-A7). EUCAST guideline was used for interpretation. The *nim* gene was investigated by PCR using specific primers. *Bacteriodes fragilis* ATCC 25285 was used as control strain.

Eight different BFG bacteria were identified, *B. fragilis* (n = 122) was the most prevalent species followed by *B. thetaiotaomicron* (n = 34). Metronidazole MIC_{50} and MIC_{90} values for all isolates were 1 mg/L and 2 mg/L, respectively. Only one isolate (0.5%), *B.fragilis*, was resistant to metronidazole with MIC 8 mg/L. A total of two isolates, metronidazole resistant *B. fragilis* and *B. uniformis* with high MIC value (4 mg/L) were *nim* gene positive.

Metronidazole displays high *in vitro* activity against BFG bacteria, it remains good candidates for empiric therapy. Although metronidazole resistance is not a risk for the time being, widespread use of metronidazole is predicted to increase the number of metronidazole-resistant BFG bacteria in the future. In order to prevent the danger or to control the resistance, it is necessary to determine the resistance profile of the bacteria to antibiotics and the resistance genes at regular intervals.

Email: nurverulgerahoo.com

ANTIMICROBIAL SUSCEPTIBILITY OF PATHOGENIC GRAM-POSITIVE ANAEROBIC COCCI, DATA OF A UNIVERSITY HOSPITAL IN TURKEY

Ulger Toprak, N.;* Akgul, O.
Marmara University, School of Medicine, Department of Microbiology, Istanbul, Turkey

Gram positive anaerobic cocci (GPAC) have been reported to develop resistance to antimicrobial drugs. The resistance of pathogens to antimicrobials and improper therapy cause poor clinical outcomes. Therefore, periodic monitoring the resistance trends of regional clinically important anaerobic bacteria is recommended. In our study, we aimed to determine the antimicrobial susceptibility profiles of clinically important GPAC.

A total of 175 non-duplicated pathogenic GPAC were collected from Marmara University Hospital between 2013 and 2017. The isolates were identified by using MALDI-TOF MS (VITEK MS; v3.0). The MICs of 11 antimicrobials were determined using agar dilution methodology (CLSI; M11-A7) and interpreted according to the breakpoints described by the EUCAST, CLSI or FDA guidelines.

The identification results showed that the collection of 175 strains consisted out of five different GPAC genus; *Parvimonas* (40%), *Finegoldia* (33.1%), *Peptoniphilus* (15.4%), *Peptostreptococcus* (9.7%), and *Anaerococcus*. All of the organisms were susceptible to meropenem, tigecycline, and metronidazole. The isolates were highly susceptible to amoxicillin/clavulonic acid (AMC), cefoxitin, and chloramphenicol, as their resistance rates to these antimicrobials were 2.3% or less. The resistance rates to penicillin, clindamycin, moxifloxacin, and tetracycline were 7.4%, 21.1%, 30.3% and %33.1, respectively. In total, 10.3% of isolates were multidrug resistant.

In conclusion, Metronidazole and tigecycline display high *in vitro* activity against GPACs and both are appropriate antimicrobials to select for empiric therapy. Meropenem was also found to be very active, but its usage should be reserved for serious mixed infections, potentially accompanied by other resistant organisms. Non-susceptibility to penicillin, AMC, which are frequently used in treatment of GPACs infection emphasize the need of periodic monitoring of their susceptibility patterns. The high rates of resistance to clindamycin, tetracycline, and moxifloxacin indicate that these antimicrobials should not be used for empirical treatment of infections without prior antimicrobial susceptibility testing.

Email: nurverulgerahoo.com

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