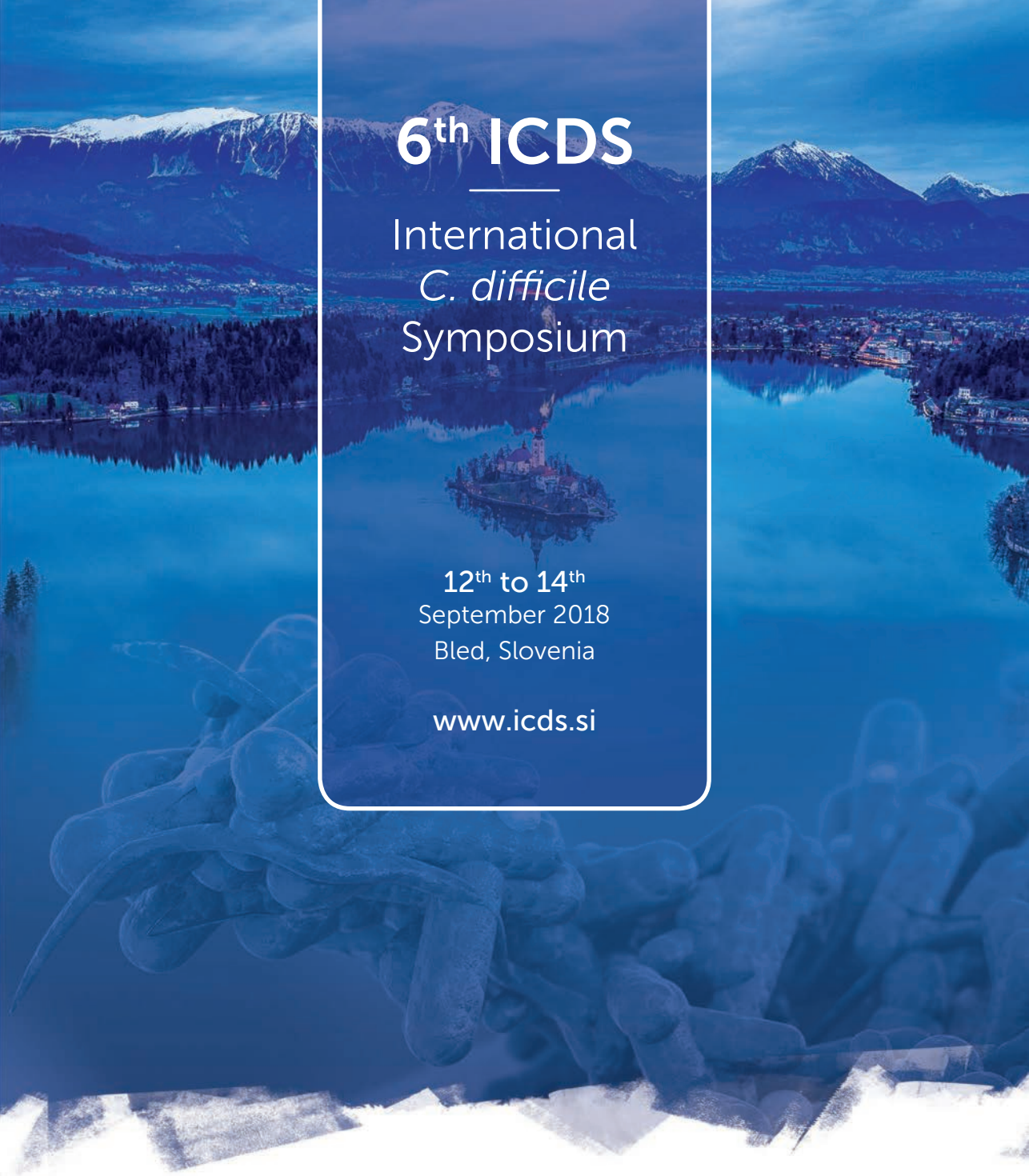


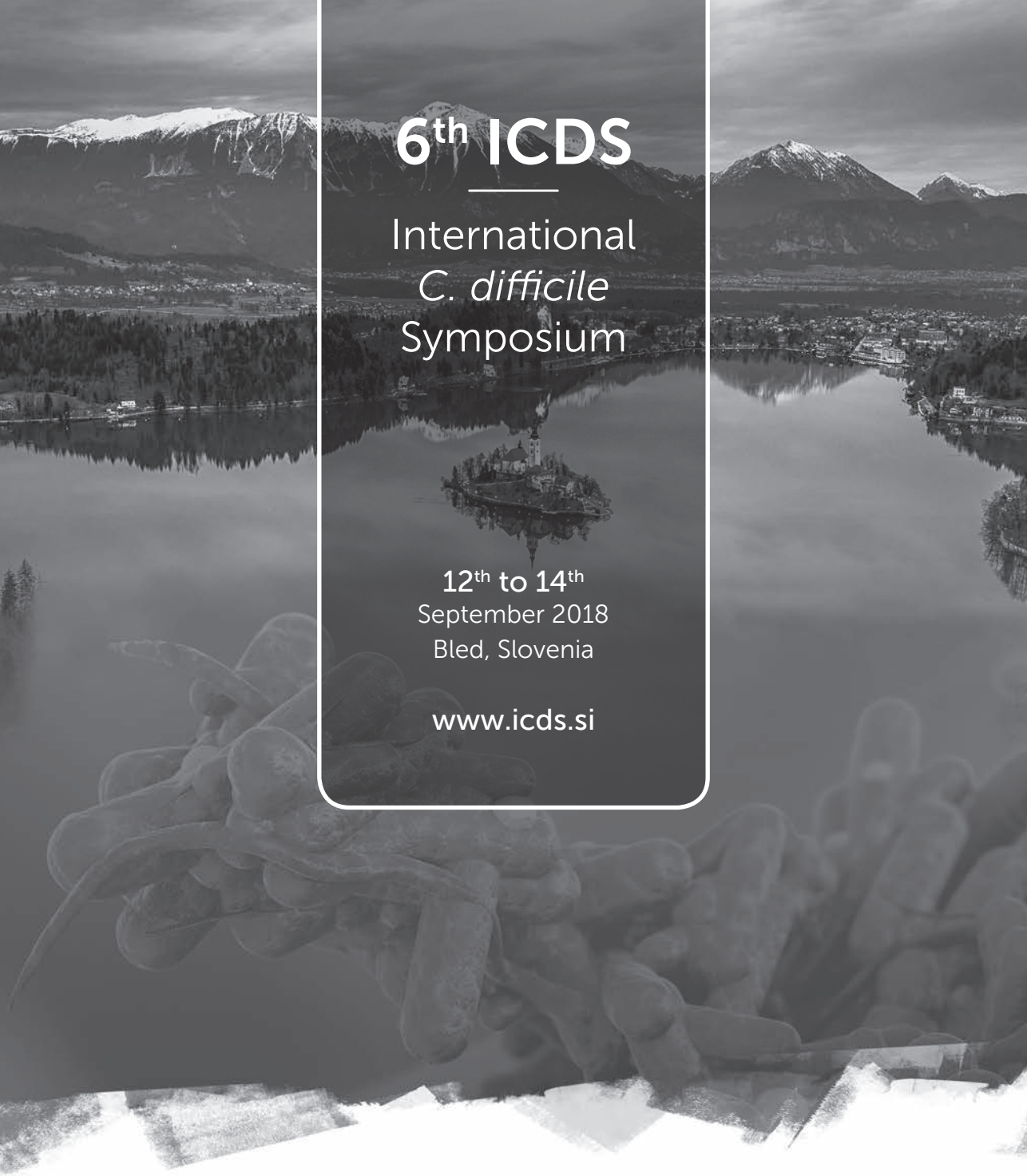
6th ICDS

International
C. difficile
Symposium

12th to 14th
September 2018
Bled, Slovenia

www.icds.si

ABSTRACT BOOK



6th ICDS

International
C. difficile
Symposium

12th to 14th
September 2018
Bled, Slovenia

www.icds.si

ABSTRACT BOOK

6th International *C. difficile* Symposium Abstract book

Zbrali in uredili: Maja Rupnik
Sandra Janežič
Oblikovanje in prelom: Uroš Zupančič s.p.
Založil: Nacionalni laboratorij za zdravje, okolje in hrano Maribor
Naklada: 240 kos

CIP - Kataložni zapis o publikaciji
Narodna in univerzitetna knjižnica, Ljubljana

579.852.13(082)
616.98:579.852.13(082)

INTERNATIONAL *C. difficile* Symposium (6 ; 2018 ; Bled)
Abstract book / 6th ICDS - International C. difficile Symposium, 12th to 14th September 2018, Bled, Slovenia ; [zbrali in uredili Maja Rupnik, Sandra Janežič]. - Maribor : Nacionalni laboratorij za zdravje, okolje in hrano, cop. 2018

ISBN 978-961-6148-18-4
1. Rupnik, Maja, 1967-
296353280

ICDS would like to acnowledge the contributions of

Platinum Sponsors

Synthetic BIOLOGICS

Pfizer

Gold Sponsors

valneva

meridian BIOSCIENCE ™
LIFE DISCOVERED. LIFE DIAGNOSED.

MERCK

Cepheid®
A better way.

Silver Sponsors

summit

ESGCD ESCMID STUDY GROUP FOR CLOSTRIDIUM DIFFICILE
European Society of Clinical Microbiology and Infectious Diseases

GenePOC™

Bronze Sponsors

Rebiotix®
Microbiota Restoration Therapy

Sponsors

TECHLAB®

r-biopharm

DaVolterra

C.diff. SURVIVORS
cdiffsurvivors.org

cdiff
cdifffoundation.org

BIOCODEX

SERES THERAPEUTICS™

ORGANIZING COMMITTEE

Rupnik, Maja (Slovenia)
Janezic, Sandra (Slovenia)
Barbut, Frederic (France)
Roberts, Adam (UK)
Wilcox, Mark (UK)
Dubberke, Erik (USA)
Johnson, Stuart (USA)
Young, Vincent (USA)
Vedantam, Gayatri (USA)
Shen, Aimee (USA)
Louie, Tom (Canada)
Kato, Haru (Japan)
Lyras, Dena (Australia)
Riley, Thomas (Australia)

CONTENTS

WELCOME

Dear colleagues and friends,

Welcome to the 6th International C. difficile Symposium.

ICDS started in 2004 as a venue providing and enhancing communication between different groups working in one way or another with this constantly surprising pathogen. At that time, the C. difficile community was mainly split into academic and clinical sections. This was also a time when sessions on C. difficile or even lectures at other large or small meetings were rather rare. Although this changed after the ribotype 027 epidemic, and the increased clinical importance of C. difficile infection was recognised, ICDS has remained a major specialized meeting for C. difficile experts and newcomers.

During the meetings that followed in 2007, 2010, 2012 and 2015, the programme reflected developments in epidemiology, emergence of epidemic strain(s), progress in our understanding of reservoirs and transmissions together with the importance of One Health, new therapeutic options, new diagnostic options, and rapid expansions in pathogenesis research after the development of methods for genetic manipulations.

Again the programme for ICDS 2018 has been selected in a way to give the state of the art for all classical topics, such as diagnostics, therapy, and infection control, and to include the new fields like host interactions with microbiota, immunology, comparative genomics and new research methodologies.

Since the last meeting our favourite pathogen was also renamed from Clostridium to Clostridioides. Both names are theoretically correct and could be used, and this is reflected in the numerous papers to be presented at the meeting.

We started in Kranjska Gora, moved to Maribor and finally found Bled to be the preferred location for subsequent meetings. We hope that you will enjoy in this exceptional environment.

On the behalf of Organizing Committee I would like to wish you a fruitful and interesting meeting.

Maja Rupnik

GENERAL INFORMATION

Congress venue
Rikli Balance Hotel, Cankarjeva cesta 4, 4260 Bled, Slovenia

Conference registration desk opening hours

Wednesday September 12th	10:00-15:00 and during coffee breaks
Thursday September 13th	8.00 to 8.30 and during coffee breaks
Friday September 14th	8.00 to 8.30 and during coffee breaks

Meals and Social Events
Lunch is provided as part of the registration fee on Thursday September 13th and Friday September 14th. Lunch will be served in the restaurant of the Rikli Balance Hotel.

Coffee Breaks will be served in front of the lecture hall and in the poster area.

Welcome reception, on Wednesday September 12th, is included in the registration and will be held in Rikli Balance Hotel.

Congress dinner on Friday September 14th is included in Registration and will be held in Grand Hotel Toplice.

Name Badges
You are required to wear your name badge during all congress scientific sessions as well as during social events.

Certificate of Attendance
Attendance certificates for participants will be provided on-site upon request, at the Registration desk.

For Speakers
Speakers are kindly requested to upload their presentation to the computer in the lecture hall. Technical assistance will be provided. Use of own computers is not encouraged.

For poster presenters
Posters will be displayed during the entire meeting. Number of your poster is given in the Poster overview table. Posters for can be displayed from Wednesday, 12th September and should be removed after the Poster session on Friday, 14th September.

E-posters
In addition to paper posters, PDF versions of posters are accessible on our website (www.icds.si)

6TH ICDS – INVITED SPEAKERS

Britton, Robert (USA)
Cammarota, Giovanni (Italy)
Crobach, Monique (The Netherlands)
Davies, Kerrie (UK)
Donskey, Curtis (USA)
Eyre, David (UK)
Fang, Ferric (USA)
Fortier, Louis-Charles (Canada)
Janoir, Claire (France)
Johnson, Stuart (USA)
Knight, Daniel (Australia)
Salgado, Paula (UK)
Smits, Wiep Klaas (The Netherlands)
Sorg, Joseph (USA)
Tamayo, Rita (USA)
Vedantam, Gayatri (USA)
Wilcox, Mark (UK)

ICDS ATTENDANCE GRANTS

Androga, Grace (University of Western Australia, Australia)

Baines, Simon (University of Hertfordshire, UK)

Coullon, Heloise (Université Paris Sud - Faculté de Pharmacie, France)

Dong, Danfeng (Shanghai Jiaotong University School of Medicine, China)

Fernandes, Nadia (University of Sheffield, UK)

Ho, Jeffery (The Chinese University of Hong Kong, Hong Kong SAR)

Jose, Shinsmon (University of Cincinnati, USA)

Leite Costa, Cecília (Federal University of Ceará, Brazil)

Levent, Belkis (MoH, General Directorate of Public Health, Turkey)

Maikova, Anna (Skolkovo Institute of Science and Technology, Russia)

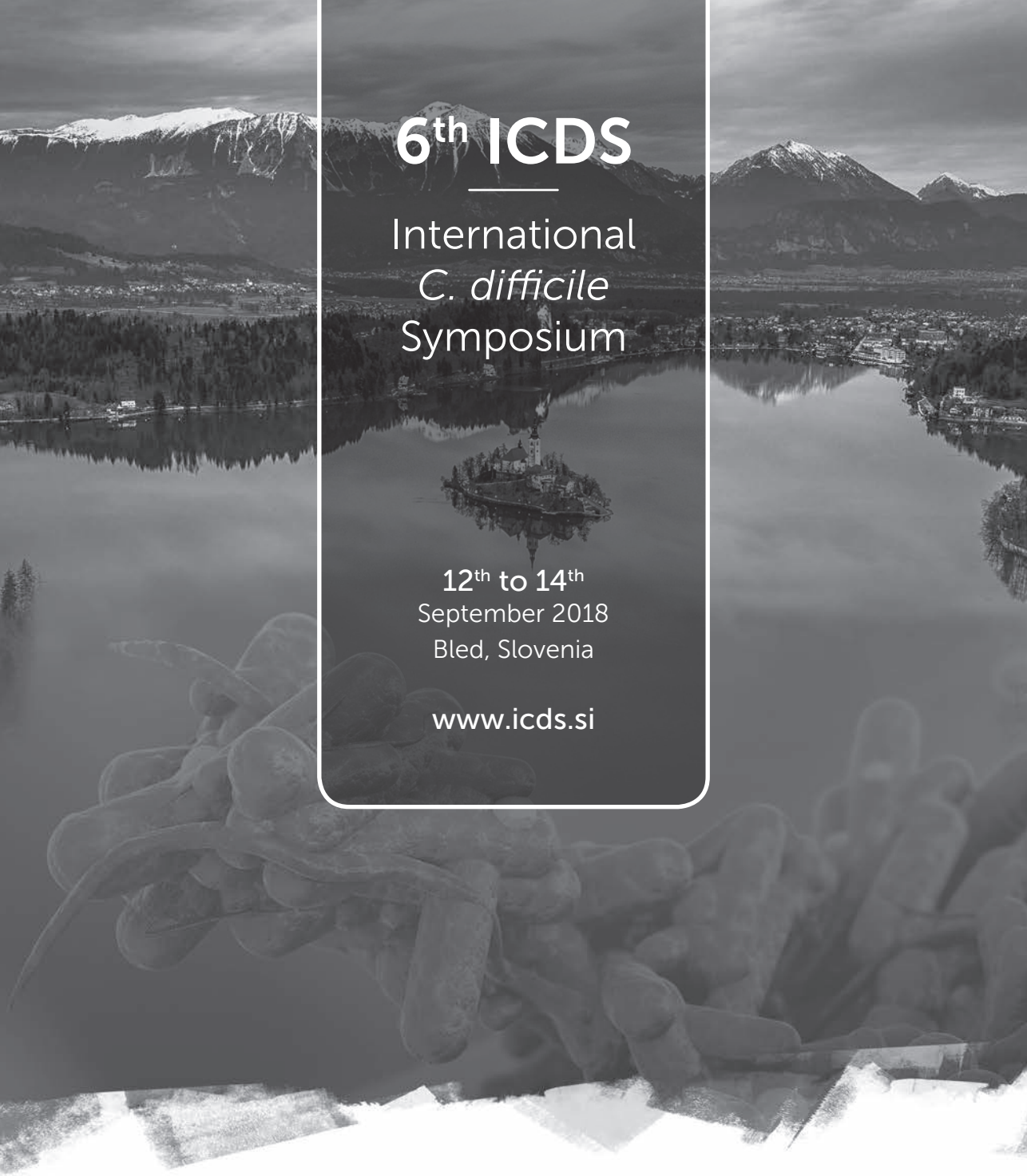
Mihaylova-Mikova, Sashka (University ""Prof. Dr. Asen Zlatarov"", Bulgaria)

Piotrowski, Michal (Medical University of Warsaw, Poland)

Romo, Mariana (CONACYT-IMSS, Mexico)

Sevilla, Eloisa (University of Zaragoza, Spain)

Wojtacka, Joanna (University of Warmia and Mazury in Olsztyn, Poland)



6th ICDS

International
C. difficile
Symposium

12th to 14th
September 2018
Bled, Slovenia

www.icds.si

ICDS
PROGRAMME

Wednesday, September 12th

15:00-16:15		**Chair: Johnson, S.**	**SESSION I: MICROBE AND HOST**
15:00-15:15		Rupnik, M.	OPENING
15:15-15:45	INV1	Britton, R.	CLOSTRIDIUM DIFFICILE ADAPTATIONS TO THE DIET: IMPLICATIONS FOR HUMAN DISEASE AND ANIMAL CARRIAGE
15:45-16:15	INV2	Vedantam, G.	C. DIFFICILE COLONIZATION AND VIRULENCE: A COMPLEX INTERPLAY BETWEEN TOXIN AND NON-TOXIN FACTORS
16:15-16:45			**COFFEE BREAK**

16:45-18:15		**Chair: Kato, H.**	**SESSION II: SHORT ORAL PRESENTATIONS I**
16:45-17:00	OP1	Dorr, M.B.	INSIGHTS FROM FIDAXOMICIN, BEZLOTOXUMAB AND SUROTOMYCIN CLINICAL TRIALS: LOOKING BEYOND THE PRIMARY ANALYSES
17:00-17:15	OP2	Kokai-Kun, J.F.	SYN-004 (RIBAXAMASE) PREVENTED CLOSTRIDIUM DIFFICILE INFECTION IN PATIENTS BEING TREATED WITH BETA-LACTAM ANTIBIOTICS
17:15-17:30	OP3	Heidebrecht, H.J.	TREATMENT AND PREVENTION OF CLOSTRIDIUM DIFFICILE INFECTION WITH FUNCTIONALIZED BOVINE ANTIBODY-ENRICHED WHEY IN A HAMSTER PRIMARY INFECTION MODEL
17:30-17:45	OP4	Mitra, S.	COMPARATIVE METAGENOMICS OF GUT MICROBIOME: RIDINILAZOLE IS ASSOCIATED WITH PRESERVATION OF MICROBIOME COMPARED WITH FIDAXOMICIN DURING TREATMENT OF CLOSTRIDIUM DIFFICILE INFECTION
17:45-18:00	OP5	Dupuy, B.	ROLE OF DEOXYCHOLATE IN INDUCTION OF CLOSTRIDIUM DIFFICILE BIOFILM FORMATION
18:00-18:15	OP6	Shaw, P.M.	GENOME WIDE ANALYSIS REVEALS HOST GENETIC VARIANTS THAT ASSOCIATE WITH REDUCTION IN CLOSTRIDIUM DIFFICILE INFECTION RECURRENCE IN PATIENTS TREATED WITH BEZLOTOXUMAB
19:30			**WELCOME RECEPTION (Hotel Rikli Balance)**

Thursday, September 13th

8:30-10:00		**Chair/moderator: Wilcox M.**	**PRO/CON DEBATE: FMT or ANTIBIOTIC MANAGEMENT**
8:30-8:55	INV3	Johnson, S.	FECAL MICROBIOTA TRANSPLANTATION (FMT) FOR C. DIFFICILE INFECTION: JUST SAY NO
8:55-9:20	INV4	Cammarota, G.	FECAL MICROBIOTA TRANSPLANTATION (FMT) FOR C. DIFFICILE INFECTION: JUST SAY YES
9:20-10:00			DISCUSSION
10:00-10:30			**COFFEE BREAK**

10:30-12:00		**Chair: Smits, W.K. SESSION III: SHORT ORAL PRESENTATIONS II**	
10:30-10:45	OP7	Vendrik, K.	CLOSTRIDIOIDES DIFFICILE PCR RIBOTYPE 023 PRESENTS AS SEVERE DISEASE WITH A COMMUNITY ONSET
10:45-11:00	OP8	Magnusson, C.	INCREASED MORTALITY IN A CLOSTRIDIUM DIFFICILE OUTBREAK DUE TO PCR RIBOTYPE 046
11:00-11:15	OP9	Golding, G.R.	MOLECULAR CHARACTERIZATION OF HEALTHCARE-ASSOCIATED CLOSTRIDIUM DIFFICILE INFECTIONS 2007-2016, CANADA
11:15-11:30	OP10	Stone, N.E.	ANALYSIS OF TOXIGENIC CLOSTRIDIUM DIFFICILE IN CANINES SUGGESTS THAT MICROBIAL MEMBERS OF THE CANINE GUT MAY PROVIDE RESISTANCE TO DISEASE
11:30-11:45	OP11	Oliveira, P.H.	EPIGENOMIC LANDSCAPE OF THE HUMAN PATHOGEN CLOSTRIDIUM DIFFICILE
11:45-12:00	OP12	Whittle, M.	IDENTIFICATION OF THE CLOSTRIDIOIDES DIFFICILE BACTERIOPHAGE RECEPTOR
12:00-13:00			**LUNCH (Hotel Rikli Balance)**
13:00-14:00			**POSTER SESSION + networking**

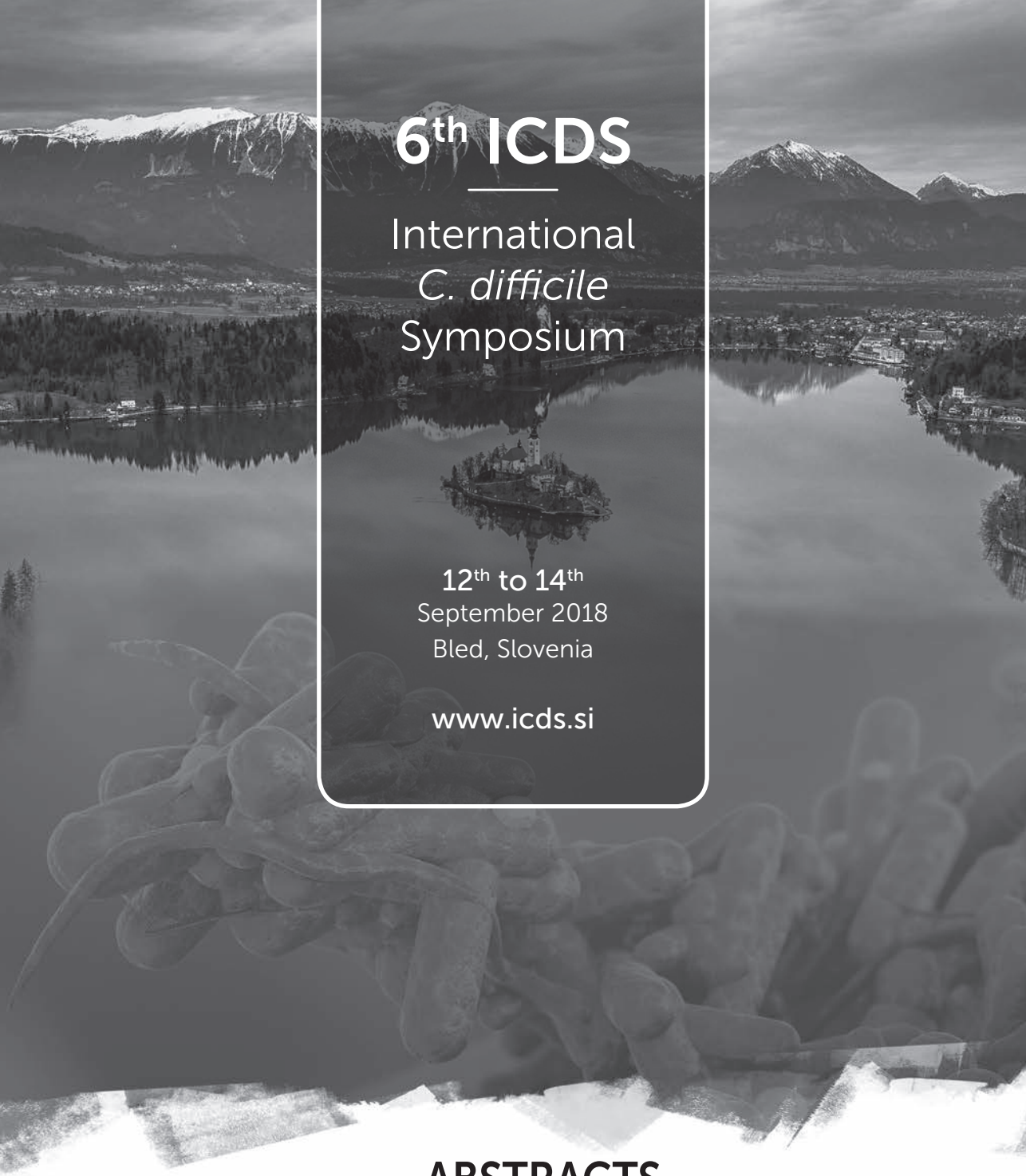
PRO/CON DEBATE: 14:00-15:30		**DIAGNOSTICS - PRO/CON PCR**	**Chair/moderator: Barbut, F.**
14:00-14:25	INV5	Fang, F.	NUCLEIC ACID AMPLIFICATION TESTING IS THE BEST APPROACH TO DIAGNOSE CLOSTRIDIUM DIFFICILE INFECTIONS
14:25-14:50	INV6	Wilcox, M.	USE OF NUCLEIC ACID AMPLIFICATION TESTS (NAATs) ALONE IS NOT SUITABLE FOR THE DIAGNOSIS OF CLOSTRIDIUM DIFFICILE INFCTION
14:50-15:30			DISCUSSION
15:30-16:00			**COFFEE BREAK**

16:00-17:00		**Chair: Shen, A. SESSION IV: EMERGING TECHNOLOGIES**	
16:00-16:30	INV7	Sorg, J.	CRISPR-CAS9 GENOME EDITING IN C. DIFFICILE
16:30-17:00	INV8	Janoir, C.	IN VIVO VISUALIZATION OF CLOSTRIDIUM DIFFICILE AND BIOFILMS
17:00			POSTER SESSION + NETWORKING + BEER TASTING

Friday, September 14th

8:30-10:00		**Chair: Vedantam, G**	**SESSION V: PATHOGENESIS - MICROBE**
8:30-9:00	INV9	Fortier, L.C.	C. DIFFICILE PHAGES
9:00-9:30	INV10	Salgado, P.	C. DIFFICILE SPORULATION: ENGULFMENT MACHINERIES AND MECHANISMS
9:30-10:00	INV11	Tamayo, R.	PHASE VARIATION IN CLOSTRIDIUM DIFFICILE: MECHANISMS AND PHENOTYPIC OUTCOMES

10:00-10:30			**COFFEE BREAK**
10:30-12:00		**Chair: Janoir, C.**	**SESSION VI: 1SHORT ORAL PRESENTATIONS III**
10:30-10:45	OP13	Maikova, A.	THE CRISPR-CAS SYSTEM OF HUMAN PATHOGEN CLOSTRIDIUM DIFFICILE: FUNCTION AND REGULATION
10:45-11:00	OP14	Sekulovic, O.	GENOME-WIDE PROFILING OF CONSERVATIVE SITE-SPECIFIC RECOMBINATION IN CLOSTRIDIUM DIFFICILE
11:00-11:15	OP15	Cuenot, E.	ROLE OF THE SERINE/THREONINE KINASE PrkC IN THE PHYSIOLOGY OF C. DIFFICILE
11:15-11:30	OP16	Coullon, H.	MURAMIC-δ-LACTAMS ARE INVOLVED IN C. DIFFICILE SPORULATION, GERMINATION AND VIRULENCE
11:30-11:45	OP17	McBride, S.M.	REGULATION OF TOXIN PRODUCTION AND SPORULATION IN CLOSTRIDIUM DIFFICILE BY THE MULTIFUNCTIONAL PROTEIN, RstA
11:45-12:00	OP18	Soutourina, O.	REGULATORY RNAS IN CLOSTRIDIUM DIFFICILE: DISCOVERY OF NEW TYPE I TOXIN-ANTITOXIN SYSTEMS ASSOCIATED WITH CRISPR ARRAYS
12:00-13:00			**LUNCH (Hotel Rikli Balance)**
13:00-14:00			**POSTER SESSION + networking**
14:00-15:30		**Chair: Dubberke, E.**	**SESSION VII CONTROL AND PREVENTION**
14:00-14:30	INV12	Donskey, C.	INFECTION CONTROL OF CLOSTRIDIUM DIFFICILE
14:30-15:00	INV13	Crobach, M.	ASYMPTOMATIC COLONISATION WITH CLOSTRIDIUM DIFFICILE
15:00-15:30	INV14	Davies, K.	THE FACTORS AFFECTING REPORTED CDI RATES
15:30-16:00			**COFFEE BREAK**
16:00-17:30		**Chair: Riley, T.**	**SESSION VIII: COMPARATIVE GENOMICS**
16.00-16.30	INV15	Knight, D.	ONE HEALTH: THE OPTIMAL PARADIGM FOR STUDYING EVOLUTION AND TRANSMISSION IN C. DIFFICILE
16:30-17:00	INV16	Eyre, D.	C. DIFFICILE TRANSMISSION IN THE HOSPITAL AND THE DIVERSE RESERVOIRS
17:00-17:30	INV17	Smits, W.K.	DECRYPTING PLASMIDS: STABLE METRONIDAZOLE RESISTANCE IN CLOSTRIDIUM DIFFICILE CORRELATES WITH A PLASMID
19.00			**CONGRESS DINNER (Grand Hotel Toplice)**



6th ICDS

International
C. difficile
Symposium

12th to 14th
September 2018
Bled, Slovenia

www.icds.si

ABSTRACTS OF INVITED AND SELECTED ORAL PRESENTATIONS

INV1

Clostridium difficile ADAPTATIONS TO THE DIET: IMPLICATIONS FOR HUMAN DISEASE AND ANIMAL CARRIAGE

Collins, James[1], Danhof, Heather[1], Britton, Robert[1]

Department of Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

As *Clostridium difficile* infections have expanded worldwide in the past 20 years, several reasons for the increase in morbidity and mortality have been explored. We have identified recent adaptations to the diet as one possible factor that has contributed to the emergence of epidemic, hypervirulent *Clostridium difficile* strains. Ribotype 027 and 078 strains of *Clostridium difficile* have acquired the ability to consume the disaccharide trehalose at much lower concentrations than other *Clostridium difficile* ribotypes. The ability to metabolize trehalose during *Clostridium difficile* infection contributes to the severity of *Clostridium difficile* infection in mice. The mechanisms by which RT027 and RT078 strains metabolize trehalose better than other ribotypes are distinct ; RT027 strains have an altered repressor or trehalose metabolism while RT078 strains have acquired a four-gene operon that enhances trehalose utilization. RT078 strains are also one of the most prevalent ribotypes found in farm animals, which may be a reservoir for human infections. In this presentation I will discuss recent evidence that supports genetic alterations in RT078 strains (and closely related ribotypes also found in farm animals) impacts the abiilty of this ribotype to utilize starches normally found in animal feed. The impact of dietary changes on the evolution of the microbiota and enteric pathogens will be discussed.

INV2

Clostridium difficile COLONIZATION AND VIRULENCE: A COMPLEX INTERPLAY BETWEEN TOXIN AND NON-TOXIN FACTORS

Gayatri Vedantam, Ph.D.

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

Clostridium difficile, the etiologic agent of antibiotic-associated diarrhea, is a Gram-positive, anaerobic, spore-forming bacterium. In the USA alone, 500,000 cases of *Clostridium difficile* infection (CDI) occur annually; these are associated with ~30,000 fatalities and a >$4 billion burden to healthcare systems. The primary *Clostridium difficile* virulence factors are toxins which are significant mediators of intestinal pathology. However, the contribution of non-toxin moieties, as well as host immune response(s), to CDI establishment and persistence are only just beginning to be appreciated. During host colonization, *Clostridium difficile* must associate with intestinal mucosa; this association can be both beneficial (potentiating colonization) and detrimental (activating immune responses). Multiple pathogen surface moieties contribute to *Clostridium difficile* establishment – these include cell-wall glycopolymers, and an array of proteinaceous components such as Surface-Layer Proteins (SLPs), flagella and pili. We and others have demonstrated significant impacts on virulence when these non-toxin factors are disrupted, suggesting that host cell intoxication alone does not fully account for the diarrheal disease spectrum associated with this pathogen. Recent clinical reports also highlight the so-called "discrepant" *Clostridium difficile* isolates, which produce low amounts of the glucosyltransferase toxins, but elicit severe disease in vivo.

Host immune status and immune responses during CDI are important drivers of disease and recovery and are now being studied in great depth. Due to the availability of analytical tools, murine models of CDI have largely been used to dissect immune responses to the pathogen and disease. These have revealed that a complex interplay between bacterial and host factors potentiates, as well as protects against, CDI. Indeed, during the CDI course, the exceptionally delicate balance just between host innate immune responses may be perturbed such that a deleterious inflammatory cascade occurs, as opposed to infection clearance. Much attention has also been paid to anti-CDI adaptive immune responses highlighted by anti-toxin-based interventions that are now available for clinical use. Adaptive immunity against non-toxin *Clostridium difficile* factors, however, can be quite variable, and a substantial effort to dissect and harness these responses – especially in the context of the SLPs - has been made over the past few years. Taken together, both toxin and non-toxin factors in *Clostridium difficile* have wide-ranging impacts on disease establishment and persistence and are therefore now considered to be appropriate candidates for interventions aimed at preventing or treating CDI. Emerging information regarding one such factor – SlpA – will be presented herein.

INV3

FECAL MICROBIOTA TRANSPLANTATION (FMT) FOR *Clostridium difficile* INFECTION: JUST SAY NO

Stuart Johnson

Hines VA Hospital, Hines, IL, Loyola University Medical Center, Maywood, IL, U.S.

Fecal microbiota transplantation (FMT) has been successful for interruption of the vicious cycle of multiple recurrences experienced by some patients with *Clostridium difficile* infection (mrCDI) and illustrates a major mechanism in the pathogenesis of CDI by correction of host colonic dysbiosis. FMT, however, is not approved by the U.S. Food & Drug Administration (FDA) or the European Medicines Agency (EMA) and there are still questions about its relative efficacy, long-term safety, standardization of the product, and the durability of response. There are now at least 7 randomized, controlled trials of FMT but only 3 in which FMT is compared to antibacterial therapy and several of these studies had serious flaws in their design. Oral vancomycin when given in a prolonged taper and pulsed regimen following a treatment course has been very effective for managing patients with mrCDI. We were able to achieve sustained cure rates of >80% in 100 consecutive mrCDI patients who were given careful follow up and an extension of the pulse phase from every other day to every third day vancomycin dosing. Fidaxomicin, like vancomycin is minimally absorbed but has less collateral damage to the indigenous host colonic microbiota and may be more effective than vancomycin for patients with mrCDI when given in a tapered and pulsed fashion after a treatment course. A recent open-label randomized controlled study of an extended-pulsed fidaxomicin regimen showed improved sustained cure rates over a standard vancomycin regimen when fidaxomicin was given twice daily for 5 days followed by once every other day for 20 days. In addition, intravenous infusion of a monoclonal anti-toxin B antibody (bezlotoxumab) when given with standard antibiotic treatment of CDI resulted and an absolute 10% decrease in CDI recurrence rates. It is possible that combining bezlotoxumab with a taper and pulsed vancomycin or fidaxomicin regimen would decrease rates of recurrent CDI even further. Rationale use of currently approved antibiotic and immune therapies can be effective for the majority of patients with mrCDI and FMT should be reserved for a small subgroup of patients who fail appropriate antibiotic management.

INV4

FECAL MICROBIOTA TRANSPLANTATION (FMT) FOR *Clostridium difficile* INFECTION: JUST SAY YES

Giovanni Cammarota

Catholic University, Faculty of Medicine, Department of Gastroenterology; Rome, Italy.

Fecal microbiota transplantation (FMT) has become widely used for the treatment of patients with recurrent bouts of *Clostridium difficile* infection (CDI). In most current settings, fresh or frozen fecal microbiota product have shown equal efficacy in the treatment of recurrent CDI. Frozen product has become preferred where available to fresh product because of advantages of convenience. All forms of application had a high cure rate, and the colonoscopic route was the most used. No relevant complications and adverse events have been documented, and the cost-effectiveness over conventional treatment has proven advantageous. Clinical trials have been conducted comparing the various forms of FMT application, their results, advantages and disadvantages. The important thing is that all the forms studied were more efficient than the treatment with antibiotics. Although several of these studies had some flaws in their design, in none of these comparator antibiotics were reaching FMT efficacy rates or were even showing to be competitive with FMT. Specific FMT protocols have been also developed for the treatment of severe CDI. Recent guidelines and consensus conferences have standardized the procedure and in some cases issued recommendations for the practical implementation of FMT centers. Despite its efficacy for the treatment of CDI recurrences, FMT is not commonly used as initial therapy. However, results achieved with a recently published small trial suggest that FMT may be an alternative to antibiotic therapy also in primary CDI, and a phase 3 trial to assess FMT as primary treatment for CDI is under way.

In conclusion, in view of the severity of CDI, it is not surprising that patients consider FMT as an alternative treatment. Education and patient involvement in the decision-making process are crucial factors for acceptance of the technique. On the other hand, the lack of regulation and institutional protocols leads to insecurity and is a barrier that needs to be overcome. The adequate use of this technique will only be feasible through the disclosure of its effectiveness, knowledge of the administration routes and acceptance of health professionals.

NUCLEIC ACID AMPLIFICATION TESTING IS THE BEST APPROACH TO DIAGNOSE *Clostridium difficile* INFECTIONS

Ferric C. Fang

University of Washington School of Medicine, Seattle, WA, USA.

The clinical spectrum of toxigenic *Clostridium difficile* ranges from asymptomatic colonization to life-threatening colitis. Thus, the diagnosis of *Clostridium difficile* Infection (CDI) requires both clinical and laboratory assessment. The most commonly used methods for the laboratory diagnosis of CDI are toxin immunoassays (EIAs) and nucleic acid amplification tests (NAATs). Toxin EIAs are less sensitive than NAATs but have a higher positive predictive value for CDI when used in unselected patients. However, a reliance on insensitive toxin EIAs can fail to detect as many as 50% of symptomatic CDI cases. This can result in delayed treatment of CDI, clinical progression and prolonged hospital length-of-stay. Although the detection of fecal toxin correlates with a higher organism burden, organism burden has a limited correlation with clinical symptoms, and even patients with severe colitis may have negative toxin EIAs. Clinical responses to oral vancomycin are comparable in patients with positive or negative toxin EIAs.

Recently published IDSA guidelines recommend a toxin EIA-based diagnostic algorithm if there are no predetermined institutional criteria for stool specimen submission. However, NAAT may be used as a standalone test if testing is limited to patients who meet clinical criteria for CDI. The NAAT approach is rapid, cost-effective, more sensitive, and avoids the underdiagnosis of CDI. When NAATs are properly employed, toxin EIAs are unnecessary. NAATs also facilitate infection control efforts, as even mildly symptomatic patients or asymptomatic carriers may contribute to *Clostridium difficile* transmission. Overdiagnosis can be minimized by limiting diagnostic testing to patients with appropriate clinical presentations.

INV6

USE OF NUCLEIC ACID AMPLIFICATION TESTS (NAATS) ALONE IS NOT SUITABLE FOR THE DIAGNOSIS OF *Clostridium difficile* INFCTION

Wilcox Mark

Affiliation(s): Leeds Teaching Hospitals, University of Leeds, Leeds, and Public Health England, UK.

NAATs have high sensitivity for (toxigenic) *Clostridium difficile* but poor specificity for *Clostridium difficile* infection (CDI). In routine practice, the positive predictive value of NAATs for CDI is as low as 54%. Currently available toxin tests are not optimal but, as part of a two-step algorithm, represent the most accurate routine approach for CDI diagnosis. Increasing evidence shows the drawbacks of relying on NAAT as single tests for CDI. These drawbacks include misdiagnosis of CDI with resulting inappropriate treatment and isolation, possible precipitation of CDI following inappropriate antibiotic treatment, false categorization of patients with recurrent diarrhoea as having recurrent CDI, failure of clinical trials, and under-estimation of the true magnitude of effect of new CDI therapeutics. NAAT do have a potential role in the diagnosis of CDI but not as routine standalone tests.

CRISPR-CAS9 GENOME EDITING IN *Clostridium difficile*

INV7

Kathleen N. McAllister & Joseph A. Sorg

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

The Clustered Regularly Interspaced Palindromic Repeats (CRISPR) are RNA-based immune systems found in bacteria and protect against invading nucleic acids (e.g., phage or plasmids). In recent years, the study of CRISPR systems has led to their application as tools for genome editing in bacteria and eukaryotes. Of the many different types if CRISPR systems, the Streptococcus pyogenes CRISPR-Cas9 system has been developed widely for its easy application for genome editing. The Cas9 nuclease is an RNA-guided, DNA nuclease that can be specifically directed to a target sequence using a short guide RNA (gRNA). This gRNA is composed of a tracer RNA and a short region that contains 20 bases of complementarity to a target sequence. Upon binding of the Cas9/gRNA complex to the target, Cas9 introduces a dsDNA break into the target sequence. In the absence of non-homologous end joining, this dsDNA break requires homologous recombination to repair the break. We have engineered a plasmid that encodes the cas9 nuclease, the gRNA and a homology region for use in DNA repair. Two cloning steps can retarget this plasmid to a new DNA sequence. We tested this plasmid against the *Clostridium difficile* pyrE gene; pyrE mutants are resistant to the toxic effects of 5-fluorouracil thereby providing an easy screening tool for development. We found that this CRISPR plasmid yielded >50% mutation frequency in the pyrE gene. When tested against a different target, selD, we found that the system yielded a ~20% mutation frequency. Since the publication of this plasmid system, we have focused on optimizing the CRISPR system by determining the 'rules' for efficient mutagenesis. We hope that we can improve the system to provide a fast and efficient avenue for genome editing in *Clostridium difficile*.

INV8

IN VIVO VISUALIZATION OF *Clostridium difficile* AND BIOFILMS

Bouttier S and Janoir C.

EA4043 Unité Bactéries Pathogènes et Santé (UBaPS), Univ. Paris-Sud, Université Paris- Saclay, 92290 Châtenay-Malabry, France

The obligate anaerobe *Clostridium difficile* is the leading cause of healthcare-associated diarrhea and has also been involved in antibiotic-associated diarrhea in the community. In patients with compromised gut microbiota (dysbiosis) due to antibiotic usage and/or underlying diseases, spores are able to germinate and the outgrowing cells may persist and multiply, leading to the colonization process. Colonization by toxigenic strains can either remain asymptomatic (carriage) or lead to *Clostridium difficile* infection (CDI), with symptoms ranging from mild diarrhea to fulminant pseudomembranous colitis. In addition, about 25% of patients experience CDI recurrence, either as reinfection or relapse. Relapse is caused by the initial strain that has persisted despite specific antibiotic treatment. Multiple relapses are suggestive of chronic infection, which raises the possibility of *Clostridium difficile* biofilm formation in vivo.

The features of gut colonization by *Clostridium difficile* are not fully understood, particularly those concerning the way in which *Clostridium difficile* associates to the mucosa. This could occur in the form of spores or of vegetative cells, embedded or not in a biofilm. Data on *Clostridium difficile* attachment to human gut tissue are scarce, and most of the information comes from studies in animal models. Several methods have been used to gain information on how *Clostridium difficile* associates to the mucosa. This overview will present the different methods used, the type of information they can provide and the main results obtained. In particular, *Clostridium difficile* has been found associated with bacterial communities within the outer mucus layer in dysbiotic mice or as micro-colonies embedded in a polysaccharidic matrix adherent to the mucus in a mono- microbial mouse model. The possible organization of *Clostridium difficile* in biofilm within the gut will be discussed considering the pathophysiology of CDI.

INV9

Clostridium difficile PHAGES

Fortier Louis-Charles

Université de Sherbrooke, Department of Microbiology and Infectious Diseases, Faculty of Medicine and Health Sciences, Sherbrooke, Quebec, Canada

Bacteriophages (phages) are fascinating viruses that parasitize bacteria in most, if not all ecosystems. Soon after their discovery a hundred years ago, phages were studied for their potential therapeutic use to fight bacterial infections. Nowadays, with the rising problem of multidrug resistance, phage therapy is even more relevant.

C. difficile causes hard to treat infections and phage therapy could represent an additional strategy in our treatment arsenal. Although recent studies suggest that phage cocktails can reduce C. difficile load in vitro and in vivo, the lack of strictly lytic phages makes typical phage therapy less attractive due to the emergence of lysogens and the potential for horizontal gene transfer. In addition, the host range of most C. difficile phages is often limited to a few strains, mostly due to the absence of a suitable phage receptor, the presence of active CRISPR-cas systems, antiphage systems, and/or endogenous prophages that limit lytic phage propagation. Hence, several factors will need to be considered for the rational design of therapeutic phage cocktails.

Quite paradoxically, several important studies have also linked phages with virulence and toxigenicity of many bacterial pathogens. In fact, most bacterial species carry one or more prophages in their genome, and C. difficile is no exception to that. Yet, their role in the biology and virulence of the bacterium is still unclear. Of note, certain phages encode genes that modulate toxin synthesis, and one phage was found to encode a functional binary toxin locus. A potential contribution of phages in quorum sensing, CRISPR-mediated immunity, superinfection exclusion, and transduction of antibiotic resistance genes has also been suggested. Studying the multiple facets of phages is therefore crucial to understand their impact on C. difficile strain diversity and virulence, and to harness their full potential in therapeutic applications, including fecal microbiota transplantation for recurrent C. difficile infections.

The past, present, and future of C. difficile phage research will be presented, and key findings related to phage biology, diversity, genomics, phage-host interactions and therapeutic applications will be discussed.

INV10

Clostridium difficile SPORULATION: ENGULFMENT MACHINERIES AND MECHANISMS

Paula S. Salgado

Institute for Cell and Molecular Biosciences and [2]Centre for Bacterial Cell Biology, Newcastle University, UK

As a strict anaerobe, *Clostridium difficile* produces dormant cell forms – spores – which allow it to survive in the aerobic environment. Importantly, the spores are the infective agent of *Clostridium difficile* infections. Spore formation is a complex differentiation programme, initiated by the master regulator Spo0A. The process begins with asymmetric cell division where the smaller compartment, the forespore, is nurtured by the larger mother cell compartment. The mother cell membrane then begins to engulf the forespore, transforming it into a free protoplast surrounded by two membranes of opposing polarity and isolated from the environment. Several studies in B. subtilis have focused on the engulfment driving forces with two key components identified: the peptidoglycan degradation machinery formed by the mother cell proteins SpoIID, SpoIIM and SpoIIP (DMP) and the so called 'zipper' complex formed by the mother cell SpoIIIAH and the forespore SpoIIQ proteins (Q:AH).

We have previously shown that, in *Clostridium difficile*, Q:AH proteins are essential for engulfment and also play a role in gene expression during sporulation. Our recent work has focussed on toxin production and general virulence of spoIIQ and spoIIIAH mutant strains in order to assess the suitability of targeting this complex as a potential therapeutic target. Recently, we investigated the role of the SpoIID, SpoIIM and SpoIIP (DMP) machinery and its interplay with Q:AH. Surprisingly, SpoIIM, the proposed machinery anchor, is not required for efficient engulfment and sporulation. We demonstrate the requirement of D/P for engulfment due to their sequential peptidoglycan degradation activity, both in vitro and in vivo. Finally, new interactions within DMP and between DMP and Q:AH suggest that both systems form a single engulfment machinery to keep the mother cell and forespore membranes together throughout engulfment.

This work sheds new light upon the engulfment process and on how different sporeformers might use the same components in different ways to drive spore formation. Understanding the molecular details of these machineries and their relation to pathogenicity are essential for the development of more targeted, novel therapeutic approaches to treating CDI.

PHASE VARIATION IN *Clostridium difficile* : MECHANISMS AND PHENOTYPIC OUTCOMES

INV11

Elizabeth Garrett[1], Dominika Trzilova[1], Brandon Anjuwon-Foster[1], Marlyn Anguelov[1], Lillian Butler[1], Ognjen Sekulovic[2], Andrew Camilli[2], and Rita Tamayo[1]

[1]University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, USA. [2]Tufts University School of Medicine, Boston, Massachusetts, USA.

The components of the bacterial cell surface play critical roles in physiology and virulence of many pathogens. These components are commonly immunogenic antigens and potential antibiotic targets. In *Clostridium difficile* the signaling molecule cyclic diguanylate (c-di-GMP) controls the production of flagella, type IV pili, and multiple additional cell surface proteins, indicating a key role for c-di-GMP in reorganizing the *Clostridium difficile* cell surface in response to the host intestinal environment. Phase variation is a means by which many bacterial species introduce phenotypic heterogeneity into the population as a strategy to ensure survival of the population in the face of changing selective pressures. Like c-di-GMP, phase variation typically modulates the production of a cell surface component that interfaces with the environment, including flagella, pili, and exopolysaccharides. Accordingly, phase variation impacts virulence and host immune recognition in many pathogens. We recently showed that flagella and toxins phase vary in *Clostridium difficile*. High throughput analysis identified a total of 7 known or putative phase variable loci in *Clostridium difficile* R20291, most of which are associated with c-di-GMP signaling, either participating in c-di-GMP metabolism or as targets of c-di-GMP regulation. A single DNA recombinase, RecV, is necessary for or involved in DNA inversions impacting phase variation of six of the seven loci. Together these findings indicate functional overlap between c-di-GMP signaling and phase variation that allows *Clostridium difficile* to coordinate global changes to the cell surface, enabling adaptation to extracellular pressures encountered in the intestinal tract. We are employing molecular genetics and biochemical techniques to determine the regulatory mechanisms of phase variation and the effects of phase variation on *Clostridium difficile* physiology. These studies may expose new targets for attenuating *Clostridium difficile* fitness in the host.

INV12

INFECTION CONTROL OF *Clostridium difficile*

Curtis Donskey, MD

Affiliation(s): Cleveland VA Medical Center, Infectious Diseases Section;[1] Case Western Reserve University School of Medicine[2]

Clostridium difficile spores are disseminated through the fecal-oral route with patients, environmental surfaces, and healthcare personnel serving as sources of transmission. Prevention of transmission is challenging because the spores survive for prolonged periods on surfaces and are resistant to killing by commonly used disinfectants and antiseptics. Basic approaches to prevent transmission focus on patients with *Clostridium difficile* infection (CDI) and include use of gloves and gowns by personnel and environmental cleaning and disinfection. Despite implementation of these basic practices, many facilities have struggled with unacceptably high CDI rates. A variety of additional approaches are therefore being studied. These approaches include: 1) addressing asymptomatic carriers of toxigenic *Clostridium difficile*; 2) antimicrobial stewardship interventions to reduce overuse of use of all antimicrobials or of specific high-risk agents; and 3) measures to reduce susceptibility to CDI using immune-based approaches or biotherapeutics.

INV13

ASYMPTOMATIC COLONISATION WITH *Clostridium difficile*

Monique Crobach

Affiliation(s): Department of Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Centre, Leiden, The Netherlands

Clostridium difficile can cause antibiotic-associated and healthcare associated infections, with symptoms ranging from mild diarrhea to a fulminant colitis. However, *Clostridium difficile* can also be silently present in the gut, without causing any symptoms. This condition is called *Clostridium difficile* colonisation (CDC). CDC can be transient or persistent, although not much is known about the natural course of *Clostridium difficile* colonisation at the moment. Reported rates of asymptomatic colonisation vary between studies due to different definitions of CDC, differences in applied laboratory methods and variable study populations. In general, lower rates have been reported in community settings, while higher rates among children, hospitalized patients and patients in long-term care facilities have been described. Depending on the toxigenic potential of the colonizing strain CDC can be subdivided into non-toxigenic CDC (ntCDC) and toxigenic CDC (tCDC). Both a disrupted microbiota including loss of primary bile acid metabolizing bacteria and lack of protective immunity are suggested to pose subjects at risk of developing CDC, although studies evaluating the microbiota in CDC subjects are scarce. On the other hand, protective immunity and specific microbiota members are thought to protect from progressing from tCDC to symptomatic *Clostridium difficile* infection (CDI). During the last years, diverse sources and risk factors for CDC have been described, and risk factors for both tCDC and ntCDC at admission to a hospital are currently studied in a multicenter study in the Netherlands. Patients with tCDC on admission to a hospital merit more attention as they can contribute to healthcare associated transmission and may have a higher risk of progressing to CDI once admitted. If interventions to prevent transmission or overcome progression to CDI in these patients could be beneficial needs to be studied further.

INV14

THE FACTORS AFFECTING REPORTED CDI RATES

Kerrie Davies

Healthcare Associated Infections Research group, Leeds Teaching Hospitals NHS trust and University of Leeds

The reported rates of *Clostridium difficile* infection (CDI) have increased in many settings, both within healthcare and in the community. However, lack of standardised *Clostridium difficile* testing is a potential confounder when comparing infection rates between countries and settings. Recent evidence shows that reported rates do not necessarily correlate with actual levels of CDI, with large numbers of missed cases in some European countries. Whilst surveillance is often encouraged, there is little information on how to interpret such reported rate data, especially considering the potential for ascertainment bias. As recently reported from a pilot study in Europe, some factors such as testing frequency, test selection criteria (e.g. patient age), type of hospital where the patient is being treated, and choice of CDI diagnostic method can affect such data. In addition, surveillance data from the US on the number of reported CDI cases from 2011 had to be adjusted to take account of the use of NAAT testing; as sensitivity analyses showed that the rate was inflated by ~2-2.5x if all laboratories used NAAT testing, compared with if no laboratories used NAAT testing for diagnosis. Despite an increased awareness of these potential influencing factors, there has been little published in the literature to date regarding the potential interplay between these factors; published studies have only used Univariate analyses. In addition, there has been little published on the potential relationship of such factors with reported seasonal variations in CDI rates. Our recent international multi-centre study demonstrated, via multivariate analyses, that low testing density is the factor that has the biggest impact on reported CDI rates. This is particularly important where low suspicion of infection, which leads to low levels of testing, may mask the true burden of disease. The choice of diagnostic method can still influence CDI reported rates however, with the use of standalone NAAT diagnosis still significantly associated with increased CDI rates, after multivariate analyses. Importantly, when all other factors, especially the testing density, were taken into consideration there was no seasonal increase in cases of CDI.

INV15

ONE HEALTH: THE OPTIMAL PARADIGM FOR STUDYING EVOLUTION AND TRANSMISSION IN *Clostridium difficile*

Knight Daniel R[1]

[1]*School of Veterinary and Life Sciences, Murdoch University, Perth, WA, Australia*

Clostridium difficile is a formidable opportunistic pathogen of humans in the developed world. This ancient species also displays a sympatric lifestyle, establishing itself in a diverse range of ecological niches external to the healthcare system. These reservoirs appear to include food, water, soil and animals, in particular, livestock such as pigs and cattle. However, in a manner analogous to human infection, excessive antimicrobial exposure, particularly cephalosporins is driving the expansion of *Clostridium difficile* in animal populations worldwide. Subsequent contamination of meat, vegetables grown in soil containing animal faeces, agricultural by-products such as compost and manure, and the environment in general, is contributing to the insidious rise of *Clostridium difficile* infection (CDI) in the community.

One Health is a philosophical approach to improving and safeguarding the health of humans, animals and the environment and, importantly, recognises that these three areas are inter-related. In this regard, CDI is the quintessential One Health issue–there is a human health problem, an animal health problem and the underappreciated factor common to both these problems, environmental contamination.

The whole-genome sequencing era continues to redefine our view of this complex pathogen. In recent years, the application of high-resolution microbial genomics in a One Health framework (encompassing clinical, veterinary and environment derived datasets) has begun to yield critical insights into the evolution and transmission of CDI. In both Europe and Australia, core genome analysis has shown *Clostridium difficile* common to humans and livestock do not form distinct populations but actually share a very recent evolutionary history. Moreover, for *Clostridium difficile* RT014 and ST11, major lineages of One Health importance, this approach has substantiated inter-species clonal transmission between animals and humans. Together these findings provide compelling evidence of either a zoonosis or anthroponosis and challenge the existing paradigm and long-held misconception that CDI is primarily a healthcare-associated infection. It is likely that animals (both human and non-human) are the true reservoir of *Clostridium difficile*. This talk will discuss various sources of *Clostridium difficile* and highlight the anthropomorphic factors that contribute to the spread of *Clostridium difficile* from the farm to the community.

INV16

Clostridium difficile TRANSMISSION IN THE HOSPITAL AND THE DIVERSE RESERVOIRS

David Eyre[1,2]

[1]Nuffield Department of Medicine, University of Oxford, Oxford, UK; [2]Big Data Institute, University of Oxford, Oxford, UK

Healthcare-associated transmission of *Clostridium difficile* is a potentially preventable source of *Clostridium difficile* infection (CDI). Patients admitted to hospital without *Clostridium difficile* acquire both detectable *Clostridium difficile* colonization and CDI in a time dependent manner. The proportion of *Clostridium difficile* acquisition arising from other cases has been studied using a range of typing methods, including more recently multiple locus variable-number tandem repeat analysis and whole-genome sequencing. In endemic settings implementing standard infection control measures multiple studies, including from the UK and USA, have reported <40% of cases arise from other cases. Other potential sources of *Clostridium difficile* in hospitals include patients colonized with *Clostridium difficile*, but without infection, both those who are asymptomatic and also patients with diarrhoea of another aetiology. The proportion of cases potentially attributable to these sources will be discussed, presenting data from the UK, Canada and the USA.

Whole-genome sequencing of isolates from consecutive clinical cases demonstrates that there is substantial genetic diversity in the strains causing disease, suggesting diverse reservoirs for CDI. Sequencing strains from across Europe suggests that different *Clostridium difficile* lineages may spread preferentially via distinct routes. Some lineages associated with healthcare-based transmission show evidence of clustering by hospital, region and country. These lineages typically display antimicrobial resistance, in particular to fluoroquinolones, which may underlie their success in healthcare, or may be a marker of wider adaptation to the healthcare environment. Other lineages show no geographic clustering. Several of these lineages have been previously associated with the food chain or wider environment. These non-clustered lineages also account for the majority of colonisation in healthy children that is genetically similar to isolates found in CDI cases.

Finally, whole-genome sequencing may provide useful surveillance data nationally and internationally. Progress towards a core-genome MLST scheme for sharing whole-genome sequencing data will be reviewed. Additionally, sequence data potentially represent a metric for assessing hospital infection control performance, data from six UK hospitals will be presented.

INV17

DECRYPTING PLASMIDS: STABLE METRONIDAZOLE RESISTANCE IN *Clostridium difficile* CORRELATES WITH A PLASMID

I.M. Boekhoud[1,2,3], B.V.H. Hornung[1,4], E. Sevilla[1,5], C. Harmanus[1], J. Corver[1,2,4], E.J. Kuijper[1,3,4], W.K. Smits[1,2,3]

[1]Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands; [2] Center for Microbial Cell Biology, Leiden, the Netherlands; [3] Netherlands Center for One Health; [4]Center for Microbiome Analysis and Therapeutics, Leiden University Medical Center, Leiden, The Netherlands; 5 Departamento de Patología Animal, Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza, Spain.

Background: Though *Clostridium difficile* has been known to carry plasmids for several decades, the role of these plasmids is largely unknown. Similarly, only a small number of plasmids have been fully sequenced and characterized. We recently identified a family of small plasmids that are similar to the pCD630 plasmid of strain 6301. Here, we extend our analysis of plasmids and plasmid function in *Clostridium difficile*.

Methods: We performed whole genome sequencing of metronidazole resistant (MIC > 2mg/L) and metronidazole susceptible (MIC < 2mg/L) isolates that were matched for PCR ribotype. Using bioinformatics, we also identified putative plasmids in publicly available paired-end whole genome sequences.

Results: A comprehensive analysis of the newly generated whole genome sequences revealed a strict correlation between metronidazole resistance and the presence of a 7-kb plasmid. None of the open reading frames on the plasmid are directly linked to previously described metronidazole resistance mechanisms. The database analysis showed that plasmid carriage is common in non-epidemic types, and that multiple plasmids can co-occur in the same strain.

Conclusions: Plasmids are rarely considered when investigating phenotypes relevant for pathogenesis. Our data, for the first time, link clinically relevant antimicrobial resistance to a highly conserved plasmid, and suggest that other plasmids with virulence-related genes circulate in the *Clostridium difficile* population.

[1] Smits WK, Weese JS, Roberts AP, Harmanus C, Hornung B. Anaerobe. 2018 Feb;49:78-84. doi: 10.1016/j.anaerobe.2017.12.005

OP1

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

Mary Beth Dorr, PhD[1], Laurent Chesnel, PhD[1], Pamela S. Sears, PhD[1]

[1]Merck & Co., Inc. Kenilworth, NJ, USA

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

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

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

OP2

SYN-004 (RIBAXAMASE) PREVENTED *Clostridium difficile* INFECTION IN PATIENTS BEING TREATED WITH BETA-LACTAM ANTIBIOTICS

John F. Kokai-Kun, Tracey Roberts, Charles Le, Heidi Whalen, Joseph Sliman.

Synthetic Biologics, Inc., Rockville, MD, USA

Background: Antibiotic treatment is the leading risk factor for development of a *Clostridium difficile* infection (CDI), and intravenous β-lactam antibiotics (penicillins and cephalosporins), which are highly excreted into the intestine through the bile, are considered high-risk antibiotics. SYN-004 (ribaxamase) is an orally administered β-lactamase which is formulated to release active enzyme at pH >5.5 which should occur about where the bile ducts drain into the upper GI. The active enzyme is designed to degrade β-lactam antibiotics excreted into the GI tract to protect the integrity of the gut microbiome and thus protect against CDI. Methods and Results: In a multinational, randomized, placebo-controlled Phase 2b clinical study in 412 patients being treated with ceftriaxone for a lower respiratory tract infection, ribaxamase reduced the incidence of CDI by 71% from 3.4% to 1% in active vs, placebo (one-sided P-value=0.045). Ribaxamase also reduced new colonization with *Clostridium difficile* and vancomycin resistance enterococci. Fecal samples for microbiome analysis were collected at three prescribed points during the study, and 16S rRNA sequencing analysis of DNA extracted from ~650 samples demonstrated that ribaxamase significantly protected the integrity of the gut microbiome as demonstrated by analysis of both α and β diversity in the two treatment groups. Whole genome shotgun sequencing was performed on a subset of ~350 samples to examine changes in the gut resistome from pre- to post-antibiotic collection points. This analysis revealed that there were significant increases in the relative abundance of certain β-lactamase and vancomycin resistance genes in the placebo group vs. the ribaxamase group. Additional analysis by qPCR confirmed the increases in these genes and appeared to support both new acquisition of antimicrobial resistance as well as expansion of existing pools of resistance within the gut resistome. Additional statistical analysis demonstrated further correlations between changes in certain AMR genes and various parameters from the clinical study.

Conclusions: These data from this clinical study support that ribaxamase has the potential to protect patients who are being treated with IV β-lactam antibiotics from CDI by protecting the integrity of the gut microbiome and also has the potential to reduce the emergence of AMR.

OP3

TREATMENT AND PREVENTION OF *Clostridium difficile* INFECTION WITH FUNCTIONALIZED BOVINE ANTIBODY-ENRICHED WHEY IN A HAMSTER PRIMARY INFECTION MODEL

Hans-Jürgen Heidebrecht[1], William J Weiss[3], Mark Pulse[3], Michael W. Pfaffl[2], Heike Kliem[2], Sacha Mann[4], Ulrich Kulozik[1], Anton Lange[5], Karina Gisch[5] and Christoph von Eichel-Streiber[5]*

*[1]TU München, Lehrstuhl für Lebensmittel- und Bio-Prozesstechnik (Chair for Food and Bioprocess Engineering), ZIEL – Institute for Food & Health, Weihenstephaner Berg 1, D - 85354 Freising-Weihenstephan, Germany; [2] Lehrstuhl für Tierphysiologie und Immunologie (Chair of Animal Physiology and Immunology), Technische Universität München, D-85354 Freising-Weihenstephan, Germany; [3]Pre-Clinical Services, UNT System College of Pharmacy, University of North Texas Health Science Center, 3500 Camp Bowie Blvd. Fort Worth, TX 76107-2699, USA; [4]BIOsys UK Ltd, 2 Queen Anne's Gate Buildings, Dartmouth Street, London SW1H 9BP; [5]tgcBIOMICS GmbH, Franz Kirsten Str.1, 55411 Bingen Germany. * corresponding author.*

Clostridium difficile Infection (CDI) is a severe complication most often of antibiotic treatment. The economic burden to the health system in the USA was estimated to approach US$4.8 billion. Relapsing and recurrent CDI is a threat to patients and physicians so that in recent years different parties all around the world launched approaches of active and passive immunizations to combat the disease. Treatment using bovine IgA antibodies for per oral pro-tection against CDI was first reported between 2000 and 2005 by a Dutch company. We recently used improved techniques of vaccine production to gain high titer primary milk from lactating cows. The main antibody content was IgA directed against TcdA and TcdB, the two Large Clostridial Toxins of *Clostridium difficile* considered their major pathogenicity factors. Addi-tionally, antibodies directed against inactivated *Clostridium difficile* cells were induced. Effective vaccination resulted in milk that potently neutralized the cytopathic effect on cultivated CHO and HT29 cells. Neutralization capacity was between >10.000 (Colostrum derived from cows 1-2 days after calving) and 100 (milk daily taken over a period of 4 months of several lacta-ting cows). An improved method of whey antibody concentrate production was established and the products thereof were used in hamster protection experiments. Three doses corresponding to neutralization factors 10.000-, 1.000- and 100-treatment were perorally applied to hamsters per treatment group (40-200mg/100g hamster of freshly resolved whey powder every 8 hours till 72 hours after infection). After 20 days observation survival rate of hamsters receiving the three doses was 100%, 50% and 75%, respectively. An according control group of 10 hamsters treated with Vancomycin (2mg/100g hamster) survived without symptoms till day ten, but nine animals finally died before day 15 (Vancomycin treatment discontinued at day 5). The ease of treatment and the promising success/survival rates of the animals prompted us to collect a couple of 10,000 liters of accordingly protective milk to be used to continue the development of bovine milk IgA concentrates and to proceed with a clinical trial in humans.

OP4

COMPARATIVE METAGENOMICS OF GUT MICROBIOME: RIDINILAZOLE IS ASSOCIATED WITH PRESERVATION OF MICROBIOME COMPARED WITH FIDAXOMICIN DURING TREATMENT OF *Clostridium difficile* INFECTION

Suparna Mitra[1,2], Caroline Chilton[1,2], Jane Freeman[2], Morag Taylor[1], Philip Quirke[1], Henry Wood[1], Richard J Vickers[2], Mark H Wilcox[1,2]

[1] University of Leeds, [2]Leeds Teaching Hospitals, Leeds, UK; [3] Summit PLC, Abingdon, UK

Background: *Clostridium difficile* infection (CDI) is a significant cause of nosocomial diarrhoea and is associated with antimicrobial-mediated dysbiosis. This may be perpetuated by antibiotic (AB) CDI treatments leading to recurrent CDI. Ridinilazole, a novel, targeted spectrum CDI antimicrobial, has been shown in a Phase 2 clinical trial to reduce the rates of CDI recurrence, when compared with vancomycin, which is likely due to its microbiota sparing action. We report stool microbiome profiling results in a longitudinal comparison during a Phase 2 randomised trial of ridinilazole (RDZ) vs fidaxomicin (FDX) for CDI treatment.

Materials/methods: Stool samples were obtained from 27 patients enrolled in the trial. Stool samples (n=154) were obtained at study entry (DM1-D1), Day 2 (D2-D3), Day 5 (D4-D5), Day 7 (D5-D7), Day 10 (D9-10), Day 12 (D11-D14), Day 25 (D15-D25), D30 (D25-30), Day 40 (D40+). DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen), and 16S V4 PCR products sequenced on an Illumina MiSeq®. Data were quality controlled and annotated (QIIME, Usearch, Greengenes, PyNAST, RDP). OTU files were imported in MEGAN for further analyses.

Results: Longitudinal comparison of patients' microbiome (154 samples) at family level showed comparable overall diversity at baseline (median simpson diversity indices 0.74 and 0.81 for RDZ and FDX group, respectively) which reduced during the antibiotic treatment for FDX (median simpson diversity indices at end-of-treatment (EOT) 0.8 and 0.65 for RDZ and FDX group, respectively). Further quantitative changes in bacterial families were assessed between baseline and EOT on all samples prior to CA use. Changes in 30 bacterial families were observed in the FDX and/or RDX; notable changes in abundance limited to approximately 10 families. Both ridinilazole and fidaxomicin, as expected, reduced the abundance of the families Peptostreptococcaceae (*Clostridium difficile*) and Clostridiaceae. Fidaxomicin was associated with reductions in abundance of Ruminococcaceae and Erysipelotrichaceae whereas increases in these families, along with the families Lachnospiraceae and Bifidobacteriaceae, were observed following ridinilazole dosing.

Conclusions: RDZ preserved gut microbiome diversity to a greater extent than FDX during CDI treatment. Differences were most marked during the treatment phase.

OP5

ROLE OF DEOXYCHOLATE IN INDUCTION OF *Clostridium difficile* BIOFILM FORMATION

Dubois Thomas[1,2], Tremblay Yannick D.N. [1,2], Hamiot Audrey [1,2], Martin-Verstraete Isabelle[1,2], Deschamps Julien[3], Monot Marc [1,2] Briandet Romain [3] and Dupuy Bruno [1,2]

[1]Laboratoire Pathogenèse des Bactéries Anaérobies, Département de Microbiologie, Institut Pasteur, 75724 Paris , France ; [2]Université Paris Diderot, Sorbonne Paris Cité, Paris, France ; [3]Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France

The biofilm, defined as structured communities of microorganisms associated with surfaces and encased in a self-produced extracellular matrix, is now recognized as causing or exacerbating numerous chronic infections. We assumed that biofilm formation might play a role in the adaptation of *Clostridium difficile* to gut colonization and/or relapse of the *Clostridium difficile* infection. Recent studies indicate that bile acids have different effects on the ability of *Clostridium difficile* to colonize gastrointestinal tract. Indeed, both cholate (CA) and deoxycholate (DCA), a secondary bile acid metabolized from CA, stimulates *Clostridium difficile* spores germination. However, DCA is toxic to vegetative *Clostridium difficile* cells. Therefore, in healthy microbiota, *Clostridium difficile* vegetative cells are exposed to the toxic effect of DCA in the colon. In contrast, during dysbiosis, transformation of CA to DCA is prevented and the CA level remains high leading to spore germination and expansion of *Clostridium difficile* in the colon. We provide experimental evidence that DCA strongly stimulates *Clostridium difficile* biofilm formation on abiotic surface reducing the sensitivity of *Clostridium difficile* to antibiotics and antimicrobial peptides. Furthermore, we demonstrated that Clostridium scindens enhanced biofilm formation and cell survival of *Clostridium difficile* in mixed biofilm by its ability to convert cholate into deoxycholate. The characterization of the matrix indicated that eDNA and proteinaceous factors are required for the integrity and/or the assembly of the DCA-induced biofilm while polysaccharides are not incorporated. In addition, several global regulators involved in the metabolic regulation are essential for the biofilm formation in response to DCA. Finally, we showed that the adaption to long-term exposure to DCA repress production of toxins and spores directly via DCA or by the DCA-induced biofilm. Altogether these results suggest that DCA is an important signal in the infectious life cycle of *Clostridium difficile* that would allow persistence of *Clostridium difficile* in the GI tract when a normal microbiota is restored, increasing the relapse's risk.

OP6

GENOME WIDE ANALYSIS REVEALS HOST GENETIC VARIANTS THAT ASSOCIATE WITH REDUCTION IN *Clostridium difficile* INFECTION RECURRENCE IN PATIENTS TREATED WITH BEZLOTOXUMAB

Judong Shen, PhD[1], Mary Beth Dorr, PhD[1], Mark H. Wilcox, MD[2], Junhua Li, MD[3], Robin Mogg, PhD[1], Devan V Mehrotra, PhD[1], Rebecca L Blanchard, PhD[1], Peter M. Shaw, PhD[1]

[1]Merck & Co., Inc., Kenilworth, NJ, [2]University of Leeds, Leeds, United Kingdom, [3]BGI-Shenzhen, Shenzhen, China

Bezlotoxumab (BEZ) and actoxumab (ACT) are monoclonal antibodies against *Clostridium difficile* toxins B and A, respectively. Patients (pts) receiving a single infusion of BEZ alone or with ACT in the MODIFY I/II trials showed a consistent reduction in the rate of rCDI over a 12-week period compared with placebo (PBO) infusion recipients. Exploratory genome wide analyses were conducted to determine if genetic variants were associated with treatment response.

DNA was extracted from blood obtained from patients (pts) who consented to genetic analysis. Genetic data were generated on an Axiom array platform (Affymetrix). Genotype imputation was performed using the 1000 Genomes Phase 3 reference data and Impute2 software after genetic quality control. Classic 4-digit HLA alleles were imputed using HIBAG. The logistic regression with likelihood ratio test was used to search for single nucleotide polymorphisms (SNPs) that were strongly associated with a treatment effect on reduction in *Clostridium difficile* infection recurrence (rCDI) relative to PBO.

The common SNP rs2516513, located in the extended major histocompatibility complex (xMHC), with a minor allele frequency of 25% in the general population, was associated with rCDI (p=3.04E-08). BEZ treated carriers of the T allele had a statistically significant reduction in rCDI compared with PBO-treated pts (absolute risk reduction (ARR) = -21.5%). The effect size of the T allele on rCDI was most prominent in pts who had $\geq$1 risk factor for rCDI (ARR = -24.6%), but was also present in pts without risk factors (ARR = -10.6%). In CC homozygous pts, rCDI rates were similar in both treatment groups and in pts at high and low risk of rCDI. In addition, two class II HLA alleles (HLA-DRB1*07:01, and HLA-DQA1*02:01) in high linkage disequilibrium (LD r^2=0.98) were also associated with a statistically significant reduction in rCDI in BEZ-treated pts.

An SNP variant rs2516513 and two HLA alleles are associated with rate of rCDI recurrence in pts treated with BEZ. The location of the associated genetic variants within xMHC suggests that a host-driven immunological mechanism plays a role in rCDI. These variants may predict pts most likely to respond to BEZ. As these are exploratory findings, the results should be replicated in an independent validation study.

OP7

Clostridioides difficile PCR RIBOTYPE 023 PRESENTS AS SEVERE DISEASE WITH A COMMUNITY ONSET

Karuna Vendrik[1], Monique Crobach[1], Helen Alexandra Shaw[2,3], Mark D. Preston[2,3], Brendan W. Wren[2], Ed Kuijper[1]

[1]Department of Medical Microbiology, Leiden University Medical Centre, Leiden, the Netherlands ; [2]Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK; [3]National Institute for Biological Standards and Controls (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, UK;

Background and aims: Clostridioides difficile PCR ribotype (RT) 023 contains all three toxin genes (TcdA, TcdB and CDT) as well as the TcdC stop codon associated with increased toxin expression, similar as in RT027 and RT078. This study assesses the clinical characteristics of patients with *Clostridium difficile* infection (CDI) due to RT023 in comparison with patients with CDI due to RT027, RT078/126, RT001, RT014/020/295 and all ribotypes (excluding RT027 and RT078/126).

Methods: Since 2009, the Dutch national surveillance program registers clinical characteristics and 30-day outcomes of hospitalized patients (>2years old) diagnosed with CDI. *Clostridium difficile* isolates are characterised at the reference laboratory. Severe CDI is defined as: fever (temperature of 38°C or higher) and leucocytosis (>15x109/L), hypoalbuminemia (<20 g/L) and/or dehydration, pseudomembranous colitis or bloody diarrhoea. A complicated course is defined as the need for surgical procedure, admission to intensive care unit or death. Also, four RT023 strains from the Netherlands were sequenced.

Results: From May 2009-February 2018, 5359 samples from 24 Dutch hospitals were ribotyped in the national surveillance program. RT023 accounted for 141 cases of CDI, a mean proportion of 2.4% (95% CI 2.0-2.8). CDI due to RT023 caused more severe disease compared to RT001, RT014/020/295 and all ribotypes, mainly due to more dehydration, hypoalbuminemia or bloody diarrhea. The number of patients with complicated courses and mortality rates were similar. There was no difference in disease severity between CDI due to RT023 and CDI due to "hypervirulent" strains RT027 or RT078/126. There was a higher mortality rate in the group of patients infected by hypervirulent strains, but CDI-related mortality was similar in the RT023, RT027 and RT078/126 groups. Community onset of symptoms was more frequently observed in the RT023 group compared to all other groups. The four RT023 strains contained the characteristic RT023 features such as a S-layer glycosylation cassette, an unique trehalose genotype and a large transposon region, similar as other European RT023 strains.

Discussion: CDI associated with *Clostridium difficile* RT023 presents with a similar severity as CDI due to the "hypervirulent" strains RT027 and RT078, but has more frequently a community onset. The CDI-related mortality is also comparable within the three groups.

OP8

INCREASED MORTALITY IN A *Clostridium difficile* OUTBREAK DUE TO PCR RIBOTYPE 046

Magnusson Cecilia[1], Bengnér Malin[2], Norén Torbjörn[3], Mernelius Sara[4], Matussek Andreas[5]

[1]Department of Infectious Diseases, Region Jönköping County, Jönköping, Sweden; [2]Office for Control of Communicable Diseases, Region Jönköping County, Jönköping, Sweden; [3]Deparment of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden;[4]Laboratory medicine, Region Jönköping County, Jönköping, Sweden; [5]Karolinska University Laboratory, Solna, Sweden.

Introduction
Clostridium difficile is a major cause of nosocomial diarrhea and pseudomembranous colitis. During 2011, an outbreak of *Clostridium difficile* infection (CDI), caused by the multi-drug resistant ribotype (RT) 046, was discovered at Högland Hospital in Eksjö, Sweden. To contain the outbreak, infection control measures including antibiotic stewardship, monthly evaluations of new CDIs, and altered cleaning procedures were initiated. In April 2012 the entire hospital was thoroughly cleaned using sporicidal agents. After these interventions there was a substantial decrease in the new number of CDIs per month. This study is a part of an extensive evaluation of the outbreak, where contributing factors to the initial spread as well as the subsequent reduction of RT 046 are being analyzed.

Methods
Clinical isolates from before, during and after the outbreak (n=338) were ribotyped and/or analyzed by whole genome sequencing. Medical patient records were evaluated to assess whether RT 046 was hospital or community acquired and whether it caused more morbidity and mortality compared to other ribotypes.

Results
Among all ribotyped strains, RT 046 was the most common (n=112) and 103/112 (92 %) of RT 046 CDIs were hospital associated. Among the RT 046 CDI cases 30 % of the patients died within the first month after their infection and 41 % of the survivors had at least one recurrence. In comparison, the patients with the six next most common RTs (n=105) died only in 7.8 % within the first month and 36% had a recurrence. There was no difference in the prescription of broadspectrum antibiotics or in comorbidity between patients with different RTs.

Conclusions
Clostridium difficile RT 046 was the dominating RT in this large nosocomial CDI outbreak. This RT seems to spread effectively in the hospital environment as a large majority (92 %) of the RT 046 cases were hospital associated. The RT 046 was more virulent compared to other strains, causing a high mortality and recurrence rate. Data from whole genome sequencing will add more information on possible virulence factors and /or clonality of the outbreak.

OP9

MOLECULAR CHARACTERIZATION OF HEALTHCARE-ASSOCIATED *Clostridium difficile* INFECTIONS 2007-2016, CANADA

George R. Golding[1], Kevin C. Katz[2], Michael R. Mulvey[1], Tim Du[1], Romeo Hizon[1], Kelly B. Choi[3], Linda Pelude[3], Kanchana R Amaratunga[3], Jun C Collet[4], Ian Davis[5], Gerald E Evans[6], Charles Frenette[7], Susy Hota[8], Pamela Kibsey[9], Joanne M Langley[10], Bonita E Lee[11], Yves Longtin[12], Dominik Mertz[13], Jessica Minion[14], Dorothy Moore[15], Murji Munira[16], Susan Richardson[17], Michelle Science[17], Andrew E Simor[18], Paula Stagg[19], Kathy N Suh[20], Geoff Taylor[21], Alice Wong[22], Nisha Thampi[23] and the Canadian Nosocomial Infection Surveillance Program.

[1]National Microbiology Laboratory, Winnipeg, MB; [2]North York General Hospital, Toronto, ON; [3]Public Health Agency Canada, Ottawa, ON; [4]BC Children's & Women's Hospitals, Vancouver, BC; [5]QEII Health Sciences Centre, Halifax, NS; [6]Kingston General Hospital, Kingston, ON; [7]McGill University Health Centre, Montréal, QC; [8]University Health Network, Toronto, ON; [9]Royal Jubilee Hospital, Victoria, BC; [10]IWK Health Centre, Halifax, NS; [11]Stollery Children's Hospital, Edmonton, AB; [12]Jewish General Hospital, Montréal, QC; [13]Hamilton Health Sciences, Hamilton, ON; [14]Regina General Hospital, Regina, SK; [15]Montreal Children's Hospital, Montréal, QC; [16]Vancouver Coastal Health, Vancouver, BC; [17]The Hospital for Sick Children, Toronto, ON; [18]Sunnybrook Health Sciences Centre, Toronto, ON; [19]Western Memorial Hospital, Corner Brook, NL; [20]The Ottawa Hospital, Ottawa, ON; [21]University of Alberta Hospital, Edmonton, AB; [22]Royal University Hospital, Saskatoon, SK; [23]Children's Hospital of Eastern Ontario, Ottawa, ON.

Information on patients admitted in Canadian hospitals acquiring *Clostridium difficile* infections (CDI) has been collected by over 60 hospitals participating in the Canadian Nosocomial Infection Surveillance Program (CNISP). This study describes the evolving molecular epidemiology of healthcare-associated (HA-) CDI from 2007 to 2016 in Canada. Hospitalized patients with HA-CDI were prospectively identified using a standard definition. *Clostridium difficile* isolation and molecular testing (pulsed-field gel electrophoresis, ribotyping, and antimicrobial susceptibilities) were performed on all eligible specimens collected March-April in adults (>18 years) and year round in pediatric patients (>1 and <18 years).

In this study, 4858 CDI cases with completed laboratory and epidemiological data were included. Overall, 50.7% of the cases were male. The majority of cases were observed in patients >64 (56.3%) years of age, followed by patients aged 18-64 (26.9%) and <18 (16.7%). NAP1 (associated with rt027) was the predominant strain type overall, accounting for 32.8% of the total *Clostridium difficile* isolates tested, followed by NAP4 (rt014 and 020) (15.2%) and NAP2 (rt072) (6.5%). Regionally, NAP1 was the predominant strain type in Central (41.1%) and Western (24.9%) Canada, but NAP4 (25.2%) was the predominant strain type in the East. The proportion of NAP1 identified in patients aged >64, 18-64, and <18 years of age were 42.0%, 29.0%, and 7.6%, respectively. In patients <18 years of age, NAP4 (27.5%) was the predominant strain type. Significant changes in *Clostridium difficile* strain types were observed from 2007-2016, including a decrease in NAP1 (41-11.7%) and NAP2 (25.3-1.8%) and increases in NAP4 (7.5-20.0%) and NAP11 (rt106) (0-16.3%).

OP9

Antimicrobial susceptibility testing revealed 33.8% of the isolates resistant to clindamycin, 0.02% to vancomycin, and 41.2% to moxifloxacin, which the latter has declined from 67.4% in 2007 to 15.9% in 2016. No resistance to metronidazole or tigecycline was observed.

Over 10 years, there has been a significant change in the molecular epidemiology of CDI, including a significant decrease in fluoroquinolone resistant strain types NAP1 and NAP2 and increases in other susceptible strains such as NAP4 and NAP11. These observations coincide with available data on reduced usage of fluoroquinolones in CNISP hospitals, which is likely a major factor.

OP10

ANALYSIS OF TOXIGENIC *Clostridium difficile* IN CANINES SUGGESTS THAT MICROBIAL MEMBERS OF THE CANINE GUT MAY PROVIDE RESISTANCE TO DISEASE

Nathan E. Stone[1], Amalee E. Nunnally[1], Victor Jimenez Jr[2], Emily K. Cope[1], Jason W. Sahl[1], Krystal Sheridan[3], Heidie M. Hornstra[1], Jacob Vinocur[4], Eric Settles[1], Fernando P. Monroy[2], Joseph D. Busch[1], Joel Terriquez[4], Paul Keim[1, 3], David M. Wagner[1]

[1]Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, USA, 86011; [2]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA, 86011; [3]Pathogen and Microbiome Institute, Translational Genomics Research Institute, Flagstaff, AZ, USA, 86001; [4]Northern Arizona Healthcare, Flagstaff Medical Center, Flagstaff, AZ, USA, 86001

Clostridium difficile infection (CDI) is an emerging public health threat. It is the most common cause of antimicrobial associated diarrhea worldwide and the leading cause of hospital-associated infections in the US, yet the burden of community-acquired infections (CAI) is poorly understood. Characterizing *Clostridium difficile* in canines is important for understanding the role canines may play in CAI and the interactions between host and pathogen. Several studies have suggested that canines carry toxigenic *Clostridium difficile* asymptomatically, which may imply that there are mechanisms responsible for resistance to *Clostridium difficile* in canines that could be exploited to combat human CDI. To assess virulence potential of canine *Clostridium difficile*, we tested whether toxins TcdA and TcdB (hereafter toxins) derived from a canine strain were capable of causing tight junction disruptions to colonic epithelial cells. Additionally, we addressed whether major differences exist between human and canine cells regarding *Clostridium difficile* toxicity by exposing them to identical toxins. We then examined the canine gut microbiome associated with *Clostridium difficile* carriage using 16S rRNA gene sequencing and looked for deviations from homeostasis as a proxy for CDI. Finally, we queried these sequences for bacterial taxa that may be associated with resistance to *Clostridium difficile* in canines. We did not observe a decreased ability of *Clostridium difficile* isolated from a canine to produce toxins or an increased ability of canine epithelial cells to resist the effects of these toxins. Additionally, canine guts were not dysbiotic in the presence of *Clostridium difficile*. These findings support asymptomatic carriage in canines and, furthermore, suggest features of the gut microbiome and/or a canine-specific immune response that protects against CDI. We identified two biologically relevant bacteria in our canine samples that may aid in *Clostridium difficile* resistance: 1) Clostridium hiranonis, which synthesizes secondary bile acids that have been shown to provide resistance to CDI in mice, and 2) Sphingobacterium faecium, which may be associated with regulating homeostasis in the canine gut via the production of sphingophospholipids. Our findings suggest that canines may be hidden reservoirs for *Clostridium difficile* and that the mechanisms of CDI resistance in the canine gut could provide insights into targeted therapeutics for human CDI.

OP11

EPIGENOMIC LANDSCAPE OF THE HUMAN PATHOGEN *Clostridium difficile*

Pedro H. Oliveira[1], Alex Kim[1], Ognjen Sekulovic[2], Theodore Pak[1], Shijia Zhu[1], Edward A. Mead[1], Gintaras Deikus[1], Marie Touchon[3,4], Martha Lewis[1], Colleen Beckford[1], Nathalie E. Zeitouni[1], Deena Altman[1,5], Elizabeth Webster[1], Irina Oussenko[1], Aneel K. Aggarwal[6], Ali Bashir[1], Gopi Patel[5], Camille Hamula[5], Shirish Huprikar[5], Richard J. Roberts[7], Rita Tamayo[8], Eric E. Schadt[1], Robert Sebra[1], Harm van Bakel[1], Andrew Kasarskis[1], Aimee Shen[2], Gang Fang[1]

[1]Department of Genetics and Genomic Sciences, Institute for Genomics and Multiscale Biology, Mount Sinai School of Medicine, New York, New York, United States of America; [2]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts, United States of America; [3]Microbial Evolutionary Genomics, Institut Pasteur, 25–28 rue du Docteur Roux, Paris, 75015, France; [4]CNRS, UMR3525, 25–28 rue du Docteur Roux, Paris, 75015, France; [5]Department of Medicine, Infectious Disease, Mount Sinai School of Medicine, New York, New York, United States of America; [6]Department of Pharmacological Sciences and Department of Oncological Sciences, Mount Sinai School of Medicine, New York, New York, United States of America; [7]New England Biolabs, Ipswich, Massachusetts, United States of America; [8]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, USA

Background and Aims. Clostridium difficile infection is the leading cause of nosocomial-acquired diarrhea and colitis across the developed world, with notable morbidity and mortality. Although significant progress has been made towards understanding its genetics and genome, the epigenome of *Clostridium difficile* and its functional impact has not been explored. In this study, we performed the first comprehensive DNA methylome analysis of *Clostridium difficile* using isolates from a hospital setting.

Methods. Herein, we mapped and characterized DNA methylomes of 36 human *Clostridium difficile* isolates using SMRT-seq and comparative epigenomics. Transcriptome profiles were obtained using RNA-seq.

Results. Not only did we observe great epigenomic diversity across isolates, strikingly, we discovered that a DNA methyltransferase with a well-defined specificity is highly conserved both across our dataset and in all the ~300 publicly available *Clostridium difficile* genomes. A comprehensive comparative epigenomic analysis highlighted multiple forms of inter- and intra-genome heterogeneity of the target sites of the conserved methyltransferase. Importantly, inactivation of the methyltransferase had a negative impact on sporulation, a key step in *Clostridium difficile* transmission, consistently supported by multi-omics data and genetic experiments. Further integrative transcriptomic analysis suggested that epigenetic regulation by DNA methylation is associated with *Clostridium difficile* host colonization and biofilm formation. The epigenomic landscape charted in this study also allowed an integrative analysis of multiple defense systems with respect to their roles in host defense and in regulating gene flux in *Clostridium difficile*.

Conclusions. Collectively, these discoveries open up a new epigenetic dimension to characterize, and potentially repress medically relevant biological processes in this critical pathogen. The experimental and analytical framework is generally applicable to the epigenomic characterization of other bacteria.

OP12

IDENTIFICATION OF THE *Clostridioides difficile* BACTERIOPHAGE RECEPTOR

Whittle, Michaella,[1] Kuehne, Sarah A.,[2] Fagan, Robert P.,[3] and Minton, Nigel P.[1]

[1]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), Centre for Biomolecular Sciences, Science Road, University Park, University of Nottingham, NG7 2RD, UK; [2]School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B5 7SA, UK; [3]Department of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield, S10 2TN, UK

The current treatment for Clostridioides difficile infection (CDI) is the use of antibiotics, namely metronidazole and vancomycin. Growing concerns over antibiotic resistance and the role of broad-spectrum antibiotics in the predisposition of the gut to *Clostridium difficile* colonisation have led to the desire for alternative therapies. Bacteriophages represent the ideal alternative as their highly specific nature would leave the natural gut microbiota of the patient unharmed. Currently there are two major barriers for phage therapy in *Clostridium difficile*, firstly there are no strictly lytic phages of *Clostridium difficile* known and secondly phages of *Clostridium difficile* have a very narrow host range, often only infecting a specific strain or PCR ribotype. This study aimed to determine the phage receptor on the surface of *Clostridium difficile* with a view to addressing this second barrier.

A novel phage, ΦCD1801, was isolated from post-anaerobic digester slurry from a Nottinghamshire sewage treatment plant and classified as Myoviridae through TEM and genomic analysis. Extensive host range testing showed the phage had broad-spectrum activity against *Clostridium difficile* PCR ribotype 078 strains, with the ability to lyse 31 out of 32 PCR ribotype 078 strains tested. However, the phage was unable to infect strains tested from a variety of other clinically relevant PCR ribotypes, including the hypervirulent PCR ribotpye 027. In order to determine the bacterial receptor for this phage, the S-layer cassette type from PCR ribotype 078, H2/6, was expressed in a phage resistant *Clostridium difficile* strain, *Clostridium difficile* 630. In the presence of the H2/6 S-layer cassette type binding of phage ΦCD1801 to *Clostridium difficile* 630 was observed. In the case where *Clostridium difficile* 630 is only expressing its own S-layer cassette type, no binding of the phage was observed.

In conclusion, the S-layer has been experimentally proven as the phage receptor for C. difficile in the case of phage ΦCD1801. Further work is required to determine whether the S-layer is also the receptor for other phages with an aim to creating a phage with a broad-spectrum host range.

OP13

THE CRISPR-CAS SYSTEM OF HUMAN PATHOGEN *Clostridium difficile*: FUNCTION AND REGULATION

Maikova Anna[1,2,3,4], Semenova Ekaterina[5], Severinov Konstantin[2,4,5], Soutourina Olga[1]

[1]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette cedex, France; [2]Skolkovo Institute of Science and Technology, Skolkovo, Russia; [3] Université Paris Diderot, Sorbonne Paris Cité, Paris, France; [4] Peter the Great St.Petersburg Polytechnic University, St. Petersburg, Russia; [5] Waksman Institute for Microbiology, Rutgers, The State University of New Jersey, Piscataway, USA.

Clostridium difficile is an anaerobic spore-forming bacterium that is the major cause of nosocomial diarrhoea associated with antibiotic therapy in Europe and worldwide. Many aspects of *Clostridium difficile* pathogenesis including molecular mechanisms of its adaptation to changing conditions inside the host still remain poorly understood. Our recent deep-sequencing data strongly suggest the importance of RNA-based mechanisms for the control of gene expression in *Clostridium difficile*. More than 200 regulatory RNAs were identified. These regulatory RNAs are involved in the control of important processes during *Clostridium difficile* infection cycle. Among the most abundant RNAs detected by this sequencing analysis were CRISPR (clustered regularly interspaced short palindromic repeats) RNAs for prokaryotic adaptive immune system against foreign invaders. During its infection cycle *Clostridium difficile* must cope with changing environments being exposed to exogenous genetic elements. Thus functional CRISPR-Cas system would be crucial for survival of this enteropathogen within bacteriophage-rich gut communities. The originality of *Clostridium difficile* CRISPR system is the presence of unusually large set of CRISPR arrays (12 in the laboratory 630 strain and 9 in the hypervirulent R20291 strain), that can be looked at as "memories" of past successful encounters with foreign genetic elements, the presence of two sets of cas genes conserved in the majority of sequenced *Clostridium difficile* genomes, the prophage location of several CRISPR arrays and the link with community-behaviour control, stress response and other defence systems. We show active expression and processing of CRISPR RNAs from multiple CRISPR arrays and provide experimental evidence for CRISPR system functionality in *Clostridium difficile*. Through genome sequencing and host-range analysis of *Clostridium difficile* phages and plasmid conjugation experiments we demonstrate the defensive functions of CRISPR-Cas system in both reference and epidemic *Clostridium difficile* strains and its adaptive function in reference strain. New aspects of the regulation of *Clostridium difficile* CRISPR-Cas system expression and function are explored as well as CRISPR-Cas system applications for genome editing and new therapeutic strategies. Altogether, these data emphasize the original features of active *Clostridium difficile* CRISPR system that might be important for *Clostridium difficile* survival during its infection cycle.

OP14

GENOME-WIDE PROFILING OF CONSERVATIVE SITE-SPECIFIC RECOMBINATION IN *Clostridium difficile*

Ognjen Sekulovic, Andrew Camilli

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts, United States of America

Background and aims: Bacteria in many ecological niches experience a common challenge in the form of unpredictable environmental fluctuations. Rapid adaptation to challenging conditions is therefore critical for survival and successful proliferation. Altering gene expression through DNA inversion is a common mechanism adopted by many bacterial species that allows quick generation of distinct subpopulations with altered fitness. We sought to explore the prevalence of conservative site-specific recombination (i.e. DNA inversion) in *Clostridium difficile* and explore its impact on bacterial biology.

Methods: We developed a computational method for detecting small genomic inversions in bacterial genomes based on analysis of high-throughput paired-end sequencing data. We applied this approach to *Clostridium difficile* followed by an experimental validation of all identified sites.

Results: In addition to two known inversion sites in *Clostridium difficile*, namely flagellar and cwpV, we detected five novel putative inversions in the ribotype-027 *Clostridium difficile* isolate R20291. All putative sites contain terminal inverted repeats and are located in intergenic regions except one which encompass a small open reading frame. We then used orientation-specific PCR to validate the inversion potential of all sites and quantified the relative orientation during exponential and stationary growth by qPCR. We further show that the master recombinase RecV is responsible for the inversion of some but not all invertible sites. Using a fluorescent gene-reporter system, we show that at least one gene from a two-component system located next to an invertible site is expressed in an on-off mode reminiscent of phase variation. Additional characterization of this two-component system suggests it plays an important role in bacterial physiology. Using the same computational approach, we detected and experimentally validated one additional inversion in two ribotype-017 strains next to a putative orphan response regulator. Another inversion was detected on a genomic island in several recently sequenced non-027 ribotype strains suggesting that in the future, new inversions are likely to be detected as additional and diverse *Clostridium difficile* isolates are sequenced.

Conclusion: Current genomic studies of *Clostridium difficile* have focused on differences such as single-nucleotide polymorphism (SNPs), deletions and insertions. Our results suggest that DNA-inversions are prevalent in *Clostridium difficile* and likely play an important role in bacterial biology. Additional mining of *Clostridium difficile* genomes using our method could reveal novel invertible sites, further underlying the tremendous potential for phenotypic heterogeneity among clonal populations of this important pathogen.

OP15

ROLE OF THE SERINE/THREONINE KINASE PRKC IN THE PHYSIOLOGY OF *Clostridium difficile*

Cuenot Elodie[1,2], Candela Thomas[3], Janoir Claire[3], García-García Tránsito[1,2], Hoys Sandra[3], Douche Thibaut[4], Matondo Mariette[4], Courtin Pascal[5], Chapot-Chartier Marie-Pierre[5], Dupuy Bruno[1,2], and Martin-Verstraete Isabelle[1,2]

[1]Laboratoire de Pathogénèse des Bactéries Anaérobies, Institut Pasteur, Paris, France; [2]Université Paris 7 – Paris Diderot, Paris, France; [3]Unité Bactéries Pathogènes et Santé EA4043, Université Paris Sud – Paris Saclay, Châtenay-Malabry, France; [4]Unité de Spectrométrie de Masse structurale et Protéomique, Département de Biologie structurale et Chimie, Institut Pasteur, Paris, France; [5]Institut National de la Recherche Agronomique, UNMR1319 Micalis, Jouy-en-Josas, France

Clostridium difficile (CD) is the leading cause of intestinal nosocomial post-antibiotic infections in adults. Exposure to certain antibiotics including cephalosporins induces dysbiosis promoting CD infection. Resistance of CD to these antibiotics is a major concern while resistance mechanisms remain poorly characterized. CD produces two toxins that cause epithelial cell damage and inflammation while additional factors associated to cell surface participate in the colonization process. During infection, CD encounters several stresses in the gut such as secondary bile salts that are toxic for vegetative cells, antimicrobial peptides released by the host, reactive oxygen and nitrogen species produced during inflammation. Pathogen survival depends on its capacity to rapidly adapt to the host environment. Protein phosphorylation is a reversible post-translational modification employed for signal transduction and regulation. Bacterial Ser/Thr kinases (STKs) regulate numerous physiological processes. In response to specific stimuli, STKs phosphorylate substrates on Ser or Thr residues to trigger the appropriate cellular response. PrkC is a particular STK that contains an extracellular part composed of several PASTA domains (Penicillin binding proteins associated with Ser/Thr kinases). The function of PrkC (CD2578) in the physiology of CD is unknown.
To investigate the role of PrkC in CD, we deleted the prkC gene by allelic chromosomal exchange in the 630Δerm background. PrkC inactivation affected the cell morphology. Moreover, a ΔprkC mutant had a reduced motility, formed more biofilms and showed an increased sensitivity to antimicrobial compounds targeting the envelope: deoxycholate, cephalosporins, cationic antimicrobial peptides and lysozyme. These phenotypes were not associated to differences in the structure of peptidoglycan. By contrast, the ΔprkC mutant released more polysaccharide II (PSII) in the supernatant suggesting a decreased deposition of this glycopolymer to the cell surface in this mutant. We are currently performing immunofluorescence studies to verify this hypothesis. These results indicate that PrkC is involved in the resistance mechanism of CD against stresses targeting the envelope and encountered in the gut during infection. This might be done by a regulation of the deposition of PSII to the surface of the bacteria.

OP16

MURAMIC-Δ-LACTAMS ARE INVOLVED IN *Clostridium difficile* SPORULATION, GERMINATION AND VIRULENCE

Coullon H.[1], Rifflet A.[2,3], Wheeler R.[2,3], Janoir C.[1], Boneca I.G.[2,3] and Candela T. [1]
[1]EA4043 Unité Bactéries Pathogènes et Santé (UBaPS), Université Paris-Sud, Université Paris-Saclay, 92290 Châtenay-Malabry, France ; [2]Institut Pasteur, Unité biologie et génétique de la paroi bactérienne, 75724 Paris, France ; [3]INSERM, Équipe Avenir, 75015, Paris

Background and aims

Spores are produced by many organisms as the result of a survival mechanism, triggered under several environmental conditions. They are multi-layered structures, one of which is a peptidoglycan layer known as the cortex. The cortex peptidoglycan has been described for several organisms, including B. subtilis and C. perfringens, but has yet to be published for C. difficile. Compared to the vegetative cell peptidoglycan, the cortex peptidoglycan possesses a unique, modified sugar called muramic-δ-lactam, synthesized by at least two enzymes: an amidase CwlD and an N-deacetylase PdaA. In this work, we analyzed the C. difficile cortex structure, we characterized the N-deacetylase involved in muramic-δ-lactam synthesis and investigated the impact of muramic-δ-lactams on C. difficile physiology and virulence.

Methods

The cortex of *Clostridium difficile* 630Δerm and pdaA mutant strains were analyzed using UHPLC coupled HRMS. Germination was assessed through optical density monitoring of spore suspensions after addition of taurocholate. Spore resistance properties were investigated by enumeration of spore suspensions after treatment with ethanol, hydrogen peroxide or heat. Sporulation was studied in liquid cultures after 72H of growth. Morphology of both strains was assessed through transmission electron microscopy.

Results

The cortex analysis revealed several differences between the B. subtilis and *Clostridium difficile* cortex structures. For instance, only 24% of muropeptides in C. difficile carried muramic-δ-lactams, compared to 50% of muropeptides in B. subtilis. Analysis of the cortex from the pdaA mutant showed minor traces of muramic-δ-lactams (0.4% of all muropeptides). Investigation of the consequences of this decrease in muramic-δ-lactams in the pdaA mutant showed a decreased sporulation rate, an altered germination, and a decreased heat-resistance. In a virulence assay, the pdaA mutant also showed a delayed virulence.

Conclusions

Surprisingly, our results suggest a much broader impact for muramic-δ-lactams in *Clostridium difficile* compared to previously characterized model organisms, such as B. subtilis. Our results highlight a novel factor linking both the germination and sporulation processes, and provide an insight into a new strategy to target C. difficile and its dissemination by targeting enzymes involved in cortex synthesis.

OP17

REGULATION OF TOXIN PRODUCTION AND SPORULATION IN *Clostridium difficile* BY THE MULTIFUNCTIONAL PROTEIN, RSTA

Adrianne N. Edwards and Shonna M. McBride

Department of Microbiology and Immunology, Emory Antibiotic Resistance Center, Emory University School of Medicine, Atlanta GA USA

The formation of dormant spores is a critical stage in the life cycle of the anaerobic pathogen, *Clostridium difficile*, permitting long-term survival outside the host. In spore-forming bacteria, the transcriptional regulator, Spo0A, activates sporulation when phosphorylated. However, the regulatory processes that control *Clostridium difficile* Spo0A phosphorylation are not conserved and largely unknown. A recently discovered regulator, RstA, positively influences sporulation and represses motility and toxin production. We investigated the function of the conserved helix-turn-helix DNA-binding domain, one of three predicted domains of the RstA protein. The DNA-binding domain is necessary for RstA-dependent repression of flagellar and toxin gene expression and rstA transcription, but not sporulation. Single nucleotide mutational analysis within an inverted repeat sequence in the rstA promoter revealed several nucleotides that are important for RstA-dependent regulation. Biotin-labeled DNA pulldown assays confirmed that RstA directly binds the rstA promoter, and that the wild-type rstA promoter is unable to bind to a truncated RstA protein lacking the DNA-binding domain. RstA also directly binds to and regulates the promoters for the sigD and tcdR genes, which encode direct regulators of toxin gene expression. Finally, expressing rstA orthologs from other clostridial species in the *Clostridium difficile* rstA mutant complements sporulation, but not toxin production, suggesting that species-specific quorum sensing regulation is required for toxin regulation, but not sporulation. Our data demonstrate that RstA is a DNA-binding transcriptional regulator that directly autoregulates its own expression and directly represses transcription of two major toxin regulators, creating a multi-tiered regulatory pathway to control toxin production.

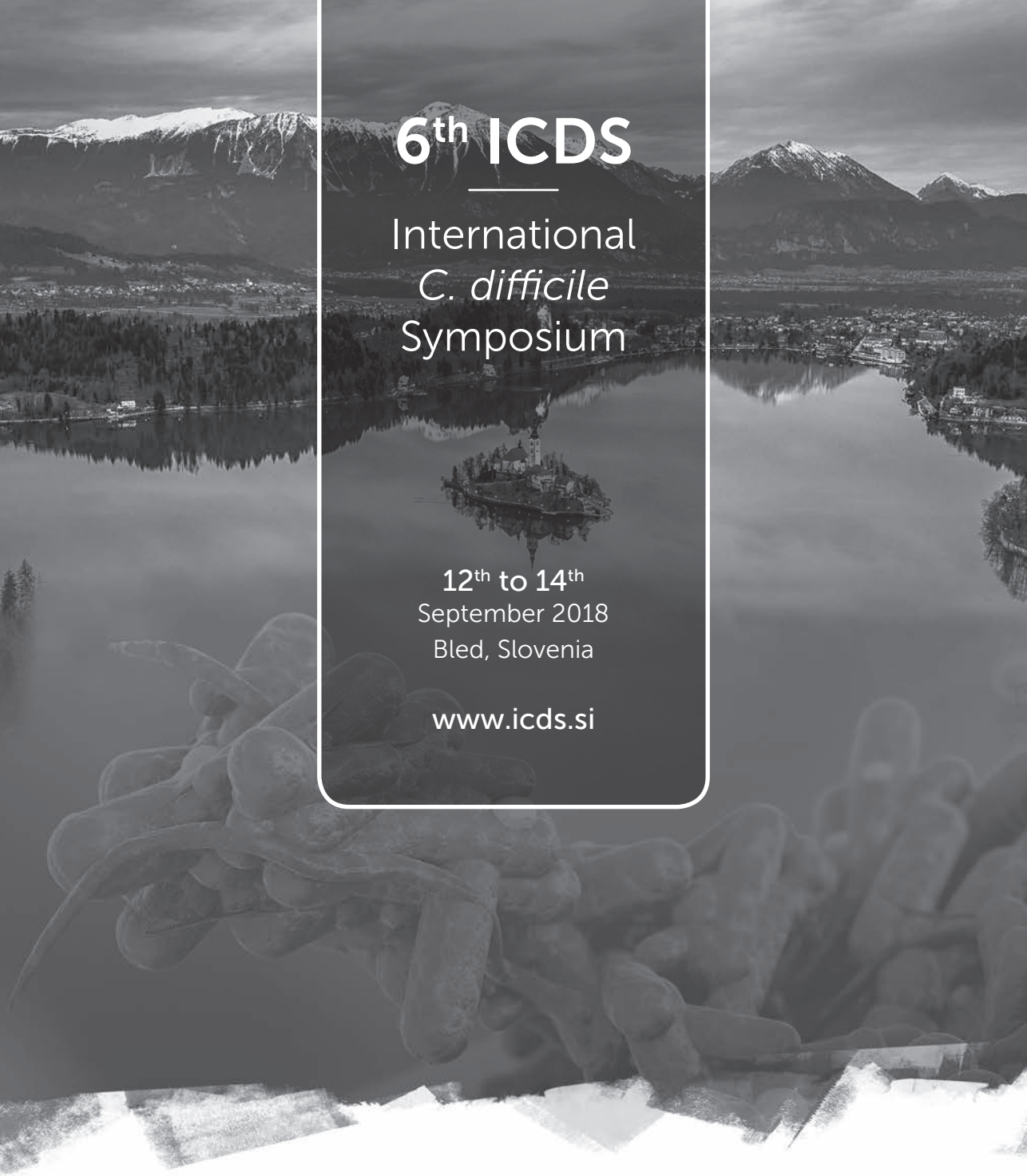
OP18

REGULATORY RNAS IN *Clostridium difficile*: DISCOVERY OF NEW TYPE I TOXIN-ANTITOXIN SYSTEMS ASSOCIATED WITH CRISPR ARRAYS

Soutourina Olga[1]

[1]*Regulatory RNAs in Clostridia group, Microbiology department, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, University Paris-Saclay, Gif-sur-Yvette, France*

During infection, bacteria reprogram their gene expression in response to environmental constraints. Non-coding RNAs (ncRNAs) play key roles in the regulation of adaptive responses. We are interested in the roles of ncRNAs in the pathophysiology of major human enteropathogen *Clostridium difficile* (CD). We have recently identified a great number (>200) and a large diversity of ncRNAs of different classes in CD. These ncRNAs might play important roles in the control of gene expression during the CD infection including metabolic adaptations, biofilm formation, stress responses, defence mechanisms and sporulation. CD must cope with foreign DNA invaders and multiple stress factors inside the host. We describe the identification of type I toxin-antitoxin (TA) systems with the first functional antisense RNAs in this pathogen. We provide a detailed characterisation and experimental validation of the overlapping convergent transcripts encoding a small membrane toxin and an antisense RNA antitoxin for selected TA modules in CD strain 630. The overexpression of toxins led to the growth arrest that could be prevented by the co-expression of antitoxin RNA. We used deep-sequencing transcriptomic and genomic data to uncover a unique co-localisation of these newly described type I TA modules with CRISPR arrays for the majority of sequenced CD strains. We have recently provided an experimental evidence of defensive function of the CD CRISPR-Cas system important for its survival within phage-rich gut communities. Co-regulation of CRISPR-Cas and type I TA by stress and biofilm-related factors further suggests a functional link with possible role in recurrent infections. This genomic link is in line with recently emerged concept of functional coupling of cell dormancy and immunity systems in prokaryotes. Two described TA modules are located within homologous prophage regions opening interesting evolutionary perspectives for their function. This is one of the first reports of the identification of functional TA systems in clostridia. Our results have a great potential for future development of new antibacterial strategies based on TA and CRISPR system applications to limit the pathogen development.



6th ICDS

International
C. difficile
Symposium

12th to 14th
September 2018
Bled, Slovenia

www.icds.si

POSTERS OVERVIEW

Presenting author	#	TITLE
Dorr, Mary Beth	P1	ENDOGENOUS SERUM IGG ANTIBODIES TO CLOSTRIDIUM DIFFICILE TOXIN B ARE ASSOCIATED WITH PROTECTION AGAINST C. DIFFICILE INFECTION RECURRENCE
Mizrahi, Assaf	P2	CLOSTRIDIUM DIFFICILE INFECTION MOUSE MODEL FOR THE ANALYSIS OF NATURAL SYSTEMIC AND MUCOSAL IMMUNITY
Mizrahi, Assaf	P3	PROSPECTIVE EVALUATION OF THE ADAPTIVE IMMUNE RESPONSE TO SLPA IN CLOSTRIDIUM DIFFICILE INFECTION
Tsai, Pei-Jane	P4	CLOSTRIDIUM DIFFICILE INDUCES AN ATP-P2X7 MEDIATED INFLAMMASOME ACIVATION
Baines, Simon	P5	PROBIOTICS TO PREVENT INDUCTION OF CLOSTRIDIUM DIFFICILE INFECTION?
Dong, Danfeng	P6	EFFECTS OF INTESTINAL COLONIZATION BY CLOSTRIDIUM DIFFICILE AND STAPHYLOCOCCUS AUREUS ON MICROBIOTA DIVERSITY IN HEALTHY INDIVIDUALS IN CHINA
Piotrowski, Michal	P7	THE INFLUENCE OF FOOD OLIGOSACCHARIDES (PREBIOTICS) ON ADHESION OF C. DIFFICILE TO THE HUMAN COLONIC EPITHELIAL CELL LINE (HT29/C1) IN VITRO: A PILOT STUDY
Moura, Ines	P8	MONITORING BACTERIAL POPULATIONS IN AN IN VITRO MODEL OF THE HUMAN COLON WITH RESOLVED CLOSTRIDIUM DIFFICILE INFECTION FOLLOWING FAECAL MICROBIOTA TRANSPLANTATION
Normington, Charmaine	P9	USE OF AN IN VITRO GUT MODEL OF RECURRENT CDI TO EVALUATE THE IMPACT OF FECAL TRANSPLANTS
Buckley, Anthony	P10	SER-109 PREVENTS RECURRENCE OF CLOSTRIDIUM DIFFICILE IN A DOSE DEPENDENT MANNER IN AN IN VITRO MODEL OF THE GI MICROBIOME
Govoni, Gregory	P11	DEVELOPMENT OF A PROBIOTIC TREATMENT FOR C. DIFFICILE INFECTIONS USING HYBRID MICROBES THAT ACT VIA MULTIPLE MODES OF ACTION
Janoir, Claire	P12	IMPACT OF SACCHAROMYCES BOULARDII CNCM I-745 ON THE IN VITRO BIOFILM OF CLOSTRIDIUM DIFFICILE
Mahnic, Aleksander	P13	C. DIFFICILE GROWTH AND CYTOTOXICITY ASSOCIATED BACTERIAL SIGNATURES IN THE IN VITRO MODULATED GUT MICROBIOTA
Siddiqui, Farida	P14	ISOGENIC BINARY TOXIN C. DIFFICILE MUTANT (CDT-) SHOWS DECREASED ADHERENCE IN VITRO COMPARED TO THE PARENT (CDT+) STRAIN
Kong, Ka Yi	P15	CLARIFYING THE MECHANISMS OF RIBOTYPE 002 VIRULENCE IN CLOSTRIDIUM DIFFICILE INFECTION
Romo, Mariana	P16	PHENOTYPIC CHARACTERIZATION OF NON-TOXIGENIC CLOSTRIDIUM DIFFICILE STRAINS ISOLATED FROM MEXICAN PATIENTS
Dawson, Lisa	P17	PLEOTROPIC REGULATORY PROTEINS SINR AND CD2215 ARE ASSOCIATED WITH BIOFILM FORMATION, MOTILITY AND SPORULATION IN C. DIFFICILE
Androga, Grace	P18	PUTATIVE VIRULENCE FACTORS IDENTIFIED IN LARGE CLOSTRIDIAL NEGATIVE, BINARY TOXIN PRODUCING C. DIFFICILE STRAINS
Hong, Stacey	P19	PHENOTYPIC CHARACTERISATION OF AN EMERGING MLST CLADE 2 LINEAGE OF CLOSTRIDIUM DIFFICILE, RIBOTYPE 251
Barwinska-Sendra, Anna	P20	STRUCTURAL CHARACTERISATION OF THE CLOSTRIDIUM DIFFICILE S-LAYER

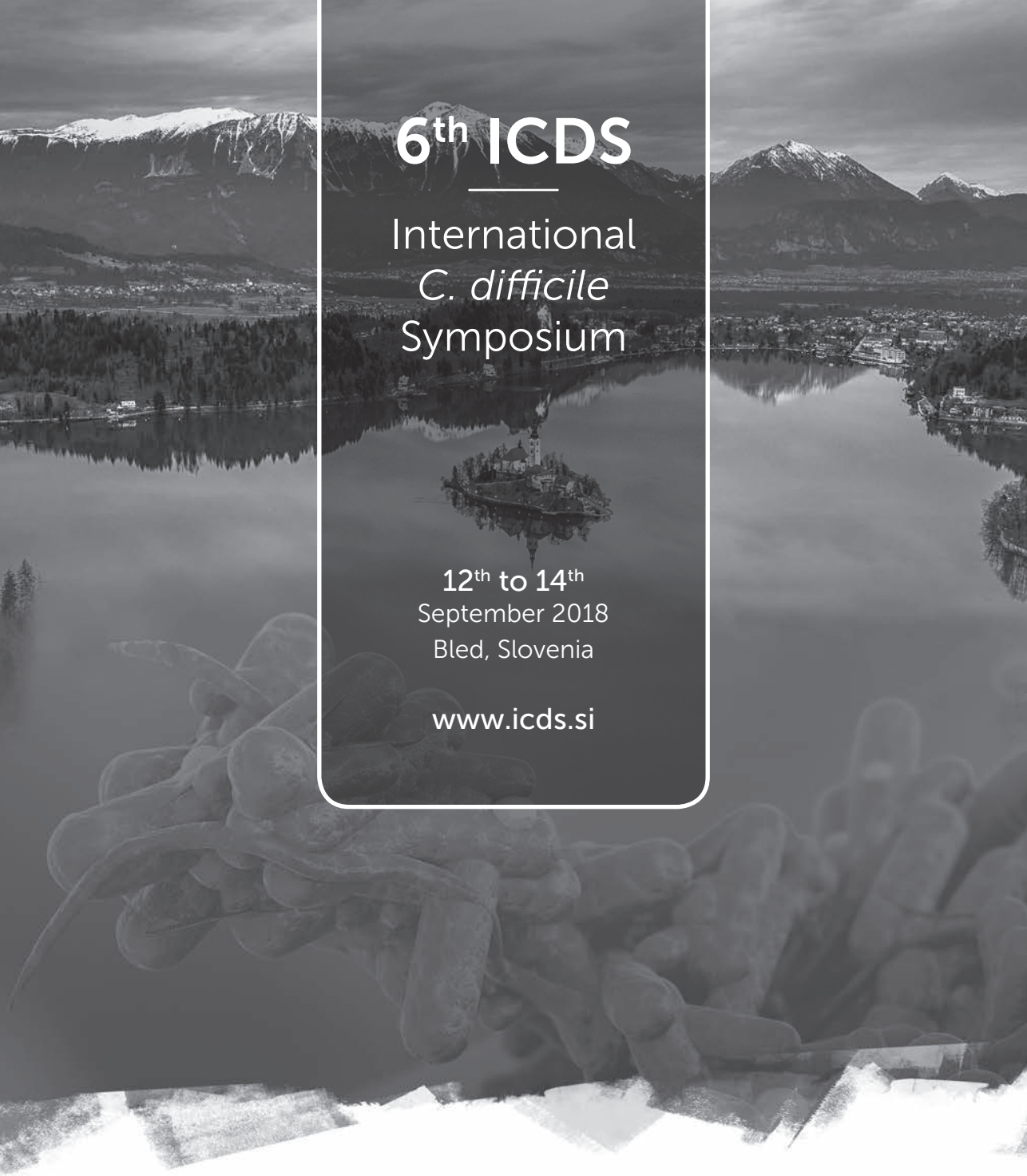
Presenting author	#	TITLE
de Castro Brito, Gerly Anne	P21	C. DIFFICILE TOXIN A INHIBITES WNT / **B**-CATENIN PATHWAY IN VIVO AND IN VITRO VIA RAC-1 INACTIVATION
de Castro Brito, Gerly Anne	P22	EFFECTS OF CLOSTRIDIUM DIFFICILE-TOXIN A AND B ON ENTERIC GLIAL CELLS
Garneau, Julian	P23	IMPACT OF THE HIGHLY PREVALENT PHI027 PROPHAGE ON THE BIOLOGY AND VIRULENCE OF THE EPIDEMIC CLOSTRIDIOIDES DIFFICILE STRAIN RIBOTYPE 027
Kelly, Abigail	P24	C. DIFFICILE PEPTIDOGLYCAN REMODELLING DURING ENGULFMENT
Harris, Hannah	P25	DEVELOPMENT OF AN IN VITRO TEST OF COLONISATION RESISTANCE TO CLOSTRIDIUM DIFFICILE IN FAECAL SAMPLES
Schwanbeck, Julian	P26	CHEMOTAXIS IN CLOSTRIDIOIDES DIFFICILE
Wang, Ziyi	P27	INVESTIGATING THE BIOLOGICAL CONTRIBUTION OF C. DIFFICILE TYPE IV PILI PROTEINS
Boekhoud, Ilse	P28	GENOME ORGANIZATION DRIVES THE TRANSCRIPTIONAL RESPONSES TO SUB-INHIBITORY CONCENTRATIONS OF POLYMERASE INHIBITORS IN CLOSTRIDIUM DIFFICILE
Corver, Jeroen	P29	PROTEOLYTIC ACTIVITY OF CLOSTRIDIUM DIFFICILE HTRA IS IMPORTANT FOR HTRA EXPRESSION LEVELS, BUT NOT FOR SPORULATION
Corver, Jeroen	P30	IDENTIFICATION AND VALIDATION OF TWO PEPTIDE MARKERS FOR THE RECOGNITION OF C. DIFFICILE MLST-1 AND MLST-11 BY MALDI-MS
Oatley, Peter	P31	S-LAYER SECRETION IN C. DIFFICILE
O'Beirne, Shauna	P32	CLOSTRIDIUM DIFFICILE CELL SURFACE BIOGENESIS
Fernandes, Nadia	P33	IDENTIFICATION OF GENES IMPLICATED IN COLONISATION AND SURVIVAL OF C. DIFFICILE IN THE GUT, USING RANDOM TRANSPOSON MUTAGENESIS
Paiva, Ana	P34	THE HISTONE-LIKE PROTEIN HUPA OF CLOSTRIDIUM DIFFICILE COMPACTS DNA
Rennhack, Kim Eileen	P35	INTERACTOMIC STUDIES OF PROTEINS INVOLVED IN THE STICKLAND FERMENTATION OF CLOSTRIDIOIDES DIFFICILE
Leite Costa, Cecília	P36	MOLECULAR CHARACTERIZATION AND COMPARATIVE STUDY OF SPORULATION OF A NEW STRAIN OF C. DIFFICILE MLST CLADE 2 ISOLATED AT A CANCER HOSPITAL IN BRAZIL.
Turk, Dušan	P37	STRUCTURAL INSIGHT INTO THE CELL WALL-ANCHORING MODULE OF CLOSTRIDIUM DIFFICILE S-LAYER PROTEINS
Harrison, Mark	P38	FACTORS AFFECTING PRODUCTION OF THE BACTERIOSTATIC COMPOUND PARA-CRESOL IN C. DIFFICILE
Ingle, Patrick	P39	GENERATION OF A SNP-FREE, FULLY ERYTHROMYCIN-SENSITIVE C. DIFFICILE 630 STRAIN USING CRISPR-CAS9 MUTAGENESIS
Poquet, Isabelle	P40	A CLOSTRIDIUM DIFFICILE BIOFILM: REMODELLING METABOLISM AND CELL SURFACE TO BUILD A 3D ARCHITECTURE
Troitzsch, Daniel	P41	INVOLVEMENT OF PERR AND ITS OPERON IN THE OXIDATIVE STRESS RESPONSE IN CLOSTRIDIOIDES DIFFICILE
Michel, Annika-Marisa	P42	ADAPTATION OF CLOSTRIDIOIDES DIFFICILE TO OSMOTIC STRESS

Presenting author	#	TITLE
Alves Feliciano, Carolina	P43	OXYGEN TOLERANCE AND REACTIVE OXYGEN SPECIES PROTECTION IN CLOSTRIDIUM DIFFICILE
Sievers, Susanne	P44	ELUCIDATION OF THE MECHANISTIC DETAILS OF CLOSTRIDIOIDES DIFFICILE´S TOLERANCE TO HIGH CONCENTRATIONS OF DIFFERENT BILE ACIDS
Peltier, Johann	P45	THE DIVERSE ROLES OF CYCLIC-DI-AMP IN CLOSTRIDIUM DIFFICILE
Kint, Nicolas	P46	DECIPHERING OF THE SIGMA B SIGNALLING ACTIVATION PATHWAY IN CLOSTRIDIUM DIFFICILE
Logan, Susan	P47	DEVELOPMENT AND TESTING OF A RECOMBINANT BCLA3 SPORE GLYCOPEPTIDE VACCINE FOR PREVENTION OF C. DIFFICILE INFECTION
Nale, Janet	P48	COMBATING CLOSTRIDIUM DIFFICILE INFECTION WITH AN OPTIMISED BACTERIOPHAGE COCKTAIL
Reigadas, Elena	P49	FECAL MICROBIOTA TRANSPLANTATION FOR RECURRENT CLOSTRIDIUM DIFFICILE INFECTION: EXPERIENCE WITH LYOPHILIZED CAPSULES
Roshan Hesari, Niloufar	P50	MECHANISMS OF ANTIBACTERIAL ACTION OF PLANT EXTRACTS AGAINST CLOSTRIDIUM DIFFICILE
Vendrik, Karuna	P51	TWO YEARS OF EXPERIENCES WITH TREATMENT OF CLOSTRIDIOIDES DIFFICILE INFECTIONS USING THE NETHERLANDS DONOR FECES BANK
Duperchy, Esther	P52	RIDINILAZOLE (RDZ) REDUCES RECURRENCE OF CLOSTRIDIUM DIFFICILE INFECTION (CDI) WITH MINIMAL IMPACT ON THE GUT MICROBIOTA
Bassères, Eugénie	P53	VISUALIZATION OF THE ASSOCIATION OF FIDAXOMICIN AND CLOSTRIDIOIDES DIFFICILE SPORES
Chapman, Sarah	P54	ISOLATION AND CHARATERISATION OF A NOVEL BACTERIOCIN THAT IS ACTIVE AGAINST CLINICALLY RELAVENT STRAINS OF C. DIFFICILE
Sambol, Susan	P55	ABSENCE OF PASSIVE TRANSFER OF TOXIN GENES FROM TOXIGENIC C. DIFFICILE (CD) TO NON-TOXIGENIC C. DIFFICILE (NTCD) STRAIN M3
Blount, Ken	P56	EVALUATING SAFETY AND EFFICACY OF MICROBIOTA-BASED THERAPIES FOR RECURRENT CLOSTRIDIUM DIFFICILE INFECTION: THE ADDED INSIGHT OF MICROBIOME PROFILING AND THE MICROBIOME HEALTH INDEX IN TWO PHASE 2 CONTROLLED TRIALS OF RBX2660
Nikolic, Natasa	P57	ORAL TEICOPLANIN FOR SUCCESSFUL TREATMENT OF RECURRENT CLOSTRIDIUM DIFFICILE INFECTION
Wu, Yuan	P58	HIGH RATE OF MULTI DRUG RESISTANT CLADE 4 CLOSTRIDIUM DIFFICILE ISOLATES FROM CHINA AND CARRIAGE OF ANTI BIOTIC RESISTANT GENES
Sevilla, Eloisa	P59	ABSENCE OF A METRONIDAZOLE RESISTANCE MARKER IN CLOSTRIDIOIDES DIFFICILE PCR-RIBOTYPE 078 ISOLATES FROM PORCINE ORIGIN
Vernon, Jonathan	P60	FLUOROQUINOLONE RESISTANCE MUTATION CONFERS A FITNESS ADVANTAGE ON CLOSTRIDIUM DIFFICILE IN A CONTINUOUS CO-CULTURE MODEL
Avberšek, Jana	P61	ANTIMICROBIAL RESISTANCE OF C. DIFFICILE ISOLATES OBTAINED FROM TWO PIG FARMS IN LONGITUDINAL STUDY
Stas, Jeroen	P62	MOLECULAR DIAGNOSTICS IN CDI: SWITCHING TO NAAT AS SCREENING METHODOLOGY?

Presenting author	#	TITLE
Bajaj-Elliott, Mona	P63	DETECTION OF TOXIN-PRODUCING CLOSTRIDIUM DIFFICILE IN THE PAEDIATRIC POPULATION AT GREAT ORMOND STREET HOSPITAL (GOSH): AN EVALUATION OF CURRENT METHODOLOGIES AND DEVELOPMENT OF A TESTING ALGORITHM.
Van Broeck, Johan	P64	ENHANCED SENSITIVITY AND ACCURACY BY LASER READING OF RAPID TESTS FOR CLOSTRIDIUM DIFFICILE GDH DETECTION
Koskinen, Juha M.	P65	GDH AND TOXIN A/B CONCENTRATIONS DO NOT DEPEND ON STOOL CONSISTENCY IN PATIENTS WITH CLINICAL SUSPICION FOR CDI
Barbut, Frédéric	P66	THE SIMOA ASSAY FOR DETECTION OF CLOSTRIDIUM DIFFICILE TOXINS HAS A BETTER SENSITIVITY THAN THE CYTOTOXICITY ASSAY
Barbut, Frédéric	P67	SUBTYPING CLOSTRIDIUM DIFFICILE PCR-RIBOTYPE 018 STRAINS BY ANALYSIS OF VIRULOME, RESISTOME, WGMLST AND MLVA
Kim, Jong Wan	P68	DETECTION OF CLOSTRIDIUM DIFFICILE BASED ON REAL-TIME PCR WITH PNA PROBE
Savolainen, Roosa	P69	EVALUATION OF A NEW RANDOM-ACCESS ANTIGEN TEST FOR THE DETECTION OF CLOSTRIDIUM DIFFICILE
Pride, Michael	P70	DIAGNOSTIC ASSAYS IN SUPPORT OF PFIZER'S PHASE 3 C. DIFFICILE VACCINE EFFICACY STUDY
Aira Gomez, Andrea	P71	CLINICAL RELEVANCE OF CT VALUE IN C. DIFFICILE INFECTION DIAGNOSIS
Mihaylova, Sashka	P72	TWO YEARS EXPERIENCE OF CLOSTRIDIUM DIFFICILE DIAGNOSIS
Krutova, Marcela	P73	THE IMPROVEMENT OF DIAGNOSTICS IN SUSPECTED C. DIFFICILE INFECTION BY IMPLEMENTATION OF A GASTROINTESTINAL PATHOGEN MULTIANALYTE TEST
Krutova, Marcela	P74	HOW IMPORTANT ARE MICROBIOLOGICAL DATA IN THE SURVEILLANCE OF CLOSTRIDIUM DIFFICILE INFECTIONS?
Reigadas, Elena	P75	ROLE OF C. DIFFICILE IN HOSPITAL ENVIRONMENT AND HEALTHCARE WORKERS
Lubarda, Jovana	P76	C. DIFFICILE PREVENTION AND MANAGEMENT: AN ASSESSMENT OF CURRENT CLINICAL PRACTICE PATTERNS OF PHYSICIANS
Silva, Rodrigo Otávio	P77	MOLECULAR EPIDEMIOLOGY OF C. DIFFICILE ISOLATES FROM A UNIVERSITY HOSPITAL OF BRAZIL
Rizzardi, Kristina	P78	C. DIFFICILE INFECTION IN SWEDEN: RESULTS FROM THE NATIONAL SURVEILLANCE PROGRAM 2009-2016
Tickler, Isabella	P79	MOLECULAR EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE PROFILES OF CLOSTRIDIUM DIFFICILE STRAINS IN THE UNITED STATES BETWEEN 2011-2017
Kato, Haru	P80	THE BURDEN OF CLOSTRIDIOIDES DIFFICILE INFECTION IN JAPAN: A PROSPECTIVE MULTI-CENTER STUDY
Connor, Mairead	P81	PREVALENCE OF CLOSTRIDIUM DIFFICILE RIBOTYPES IN NORTHERN IRELAND 2009 – 2018
Anttila, Veli-Jukka	P82	CLOSTRIDIUM DIFFICILE INFECTION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: A CASE CONTROL STUDY

Presenting author	#	TITLE
Murabata, Mayumi	P83	INTESTINAL COLONIZATION OF CLOSTRIDIOIDES DIFFICILE OF IN PEDIATRIC INFLAMMATORY BOWEL DISEASE PATIENTS IN JAPAN
Putsathit, Papanin	P84	EPIDEMIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF *CLOSTRIDIUM DIFFICILE* IN PIGLETS IN THAILAND
Kim, Heejung	P85	A NATIONWIDE STUDY OF MOLECULAR EPIDEMIOLOGY AND ANTIBICROBIAL RESISTANCE OF C. DIFFICILE IN SOUTH KOREA
Collins, Deirdre	P86	INVESTIGATING CLOSTRIDIUM DIFFICILE INFECTION IN WESTERN AUSTRALIA USING LINKED DATA
Pai, Hyunjoo	P87	PCR-RIBOTYPE VARIABILITY OF CLOSTRIDIUM DIFFICILE STRAINS FROM THE PATIENTS WITH HOSPITAL-ACQUIRED C. DIFFICILE INFECTIONS (HACDI), COMMUNITY-ACQUIRED CDI (CACDI), TOXIGENIC COLONIZATION AND NON-TOXIGENIC COLONIZATION.
Regev-Yochay, Gili	P88	CLOSTRIDIUM DIFFICILE (CD) CARRIERS: RISK FACTORS AND INCIDENCE OF CD INFECTIONS (CDI)
Grilc, Eva	P89	A TIME SERIES ANALYSIS OF RESPIRATORY SYNCITIAL VIRUS (RSV) AND ITS POSSIBLE ASSOCIATION WITH CLOSTRIDIUM DIFFICILE INFECTIONS (CD)
Lim, Su-Chen	P90	EMERGENCE OF PREDOMINANTLY COMMUNITY-ASSOCIATED CLOSTRIDIUM DIFFICILE RT 012 IN WESTERN AUSTRALIA: RISK FACTORS AND RELATEDNESS TO STRAINS FROM ASIA
Lim, Su-Chen	P91	WHOLE-GENOME SEQUENCING REVEALS POTENTIAL SPREAD OF CLOSTRIDIUM DIFFICILE BETWEEN HUMANS, FOODS AND THE ENVIRONMENT OF WESTERN AUSTRALIA
Niwa , Hidekazu	P92	WHOLE-GENOME ANALYSIS OF CLOSTRIDIOIDES DIFFICILE STRAINS ISOLATED FROM HORSES IN JAPAN
Bolea, Rosa	P93	MOLECULAR CHARACTERISATION AND ANTIMICROBIAL RESISTANCE PATTERNS IN C. DIFFICILE ISOLATED FROM THE ENVIRONMENT, HUMANS, AND OTHER ANIMAL SPECIES ORIGINATED FROM THE IBERIAN PENINSULA
Kim, Ha-Young	P94	PREVALENCE AND CHARACTERIZATION OF CLOSTRIDIUM DIFFICILE FROM DOGS AND CATS IN KOREA
Wojtacka, Joanna	P95	CLOSTRIUM DIFFICILE IN HONEY
Maurischat, Sven	P96	CLOSTRIDIUM DIFFICILE CONTAMINATION OF GERMAN RETAIL FOOD
Maurischat, Sven	P97	ZERO PREVALENCE OF CLOSTRIDIUM DIFFICILE IN GERMAN WILD GAME
Rabold, Denise	P98	ARE PET OWNERS AT INCREASED RISK OF C. DIFFICILE INFECTION?
Perumalsamy, Sicilia	P99	HIGH PREVALENCE OF CLOSTRIDIUM DIFFICILE IN SOIL, MULCH AND LAWN SAMPLES FROM WESTERN AUSTRALIAN (WA) HOSPITALS
Granata, Guido	P100	DOES NAAT TEST USE INCREASE THE RATE OF C. DIFFICILE INFECTION DIAGNOSIS?
Janezic, Sandra	P101	CAN SHOE SOLES CONTRIBUTE TO C. DIFFICILLE DISSEMINATION IN HOSPITAL ENVIRONMENT?
Janezic, Sandra	P102	WHOLE GENOME ANALYSIS OF CLOSTRIDIUM DIFFICILE PCR RIBOTYPE 150 ISOLATED FROM HUMANS, ANIMALS, ENVIRONMENT AND FOOD

Presenting author	#	TITLE
Tkalec, Valerija	P103	POTATOES SAMPLED ACROSS EUROPE ARE COMMONLY CONTAMINATED WITH CLOSTRIDIUM DIFFICILE
Jose, Shinsmon	P104	OBESITY-ASSOCIATED GUT MICROBIOTA ENHANCES CLOSTRIDIUM DIFFICILE INFECTION IN MICE
Rupnik, Maja	P105	VARIOUS WAYS OF INTERACTIONS BETWEEN CLOSTRIDIUM DIFFICILE AND GUT MICROBIOTA
Bijlani, Akash	P106	HOSPITAL COST SAVINGS USING ULTRASENSITIVE SINGLE MOLECULE COUNTING FOR DETECTION OF CLOSTRIDIUM DIFFICILE TOXINS A AND B



6th ICDS

International
C. difficile
Symposium

12th to 14th
September 2018
Bled, Slovenia

www.icds.si

ABSTRACTS OF POSTER PRESENTATIONS

P1

ENDOGENOUS SERUM IGG ANTIBODIES TO *Clostridium difficile* TOXIN B ARE ASSOCIATED WITH PROTECTION AGAINST *Clostridium difficile* INFECTION RECURRENCE

Mary Beth Dorr, PhD[1], Ian R. Poxton, PhD, DSc[2], Judong Shen, PhD[1], Radha Railkar, PhD[1], Dalya Guris, MD[1], Ciaran P. Kelly, MD[3]

[1]Merck & Co., Inc., Kenilworth, NJ; [2]University of Edinburgh, Edinburgh, United Kingdom; [3]Gastroenterology, Beth Israel Deaconess Medical Center, Boston, MA

MODIFY I/II were global trials of the efficacy and safety of bezlotoxumab (BEZ), a monoclonal antibody (mAb) against *Clostridium difficile* toxin B, alone and with actoxumab (ACT), a mAb against C. difficile toxin A. In participants (pts) treated for *Clostridium difficile* infection (CDI) BEZ was superior to placebo (PBO) at preventing recurrent CDI (rCDI). Addition of ACT did not improve efficacy. We explored potential biomarkers for rCDI risk in PBO-treated pts by measuring endogenous IgG Abs against *Clostridium difficile* toxins A and B (eAb-A, eAb-B).

Serum eAb titers were measured by electrochemiluminescence immunoassay in samples collected pre-dose (Day 1), at Week 4, and Week 12. Titers were reported as <1:1000, 1:1000, 1:5000, 1:25000, and ≥1:125000. As there is no clearly defined immunological surrogate of efficacy for rCDI tied to a specific eAb-A or eAb-B level, values were arbitrarily categorized as low (≤1:1,000), medium (1:5000), or high (≥1:25000). The rCDI rate was summarized by eAb category by time point. Correlations were assessed by 2-sided Cochran-Armitage trend test.

Approximately 97% of PBO-treated pts in the mITT population were included in the analysis for eAb-A (n=758) and eAb-B (n=754). On Day 1, 37.5% and 38.3% of pts had low titers of eAb-A and eAb-B, respectively. The proportion of pts with low eAb-A and eAb-B titers decreased over time. There was no evident correlation between eAb-A titers and the rCDI rate at any time point. A correlation (P=0.001) was observed for the proportion of pts who experienced rCDI and eAb-B titer category, with the highest rate of rCDI observed in pts with low Day 1 eAb-B titers and the lowest rate of rCDI in pts with high Day 1 eAb-B titers. A correlation was also observed for eAb-B Week 4 titers (P=0.038).

The rise in eAb-A and eAb-B titers over time is consistent with a convalescent humoral immune response to toxins A and B following CDI. A lack of correlation between eAb-A titers and rCDI is consistent with lack of efficacy of ACT in prevention of rCDI. Association of higher eAb-B titers with lower risk of rCDI is consistent with efficacy of BEZ. Despite the inverse correlation between eAb-B titers and rCDI, the rCDI rate was 22.1% in pts with high Day 1 eAb-B titers. Thus, eAb-B titers are unlikely to improve predictive value over clinical and demographic characteristics (eg, advanced age, compromised immunity) for rCDI risk.

P2

Clostridium difficile INFECTION MOUSE MODEL FOR THE ANALYSIS OF NATURAL SYSTEMIC AND MUCOSAL IMMUNITY

Assaf Mizrahi[1,2], Gauthier Péan de Ponfilly [1,2], Sandra Hoys[1], Amandine Caillault[1], Sylvie Lambert[1], Claire Janoir[1], Séverine Péchiné[1], Alban Le Monnier[1,2]

[1] EA 4043 „Unité Bactéries Pathogènes et Santé" (UBaPS), Univ. Paris-Sud, Université Paris-Saclay, 92290 Châtenay-Malabry, France ;[2] Service de Microbiologie clinique et dosage des anti-infectieux, Groupe Hospitalier Paris Saint Joseph, 75014 Paris, France

Background and aims: *Clostridium difficile* infection (CDI) remains a major concern as community or healthcare-associated infections. Various studies have highlighted the importance of the host immune response in CDI outcome. In animal models, immunization by the flagellin FliC or the precursor of the S-layer: SlpA led to the synthesis of specific antibodies with a partial protective effect on CDI. The objectives of this work were to determine the physiological kinetics and conditions of appearance of serum IgG, IgM antibodies and colonic IgA directed against the toxins: TcdA and TcdB as well as against FliC and SlpA in a naive host after a first encounter with *Clostridium difficile*.

Methods: We conducted an animal assay based on an orogastric challenge after an antibiotic pretreatment by 10^5 spores of the 630ΔErm strain in a C57Bl/6 model of infection. A group was followed after challenge clinically and on colonization level during 28 days. Blood samples were performed at D-6, D7, D14, D21 and D28. A second group was reinfected at D28 with supplementary blood samples at D42 and D69. Caecal contents were collected at D69. A control group was challenged by a non-toxigenic strain ATCC43602.

Results: We observed symptomatic aspects of CDI and a weight loss in mice challenged by the 630ΔErm strain. Mice were colonized by *Clostridium difficile* until D14. The animals that were neither colonized nor symptomatic were excluded of the study. We observed an appearance of IgM directed against all the tested antigens from D2 with a significant increase until D21. We also observed an appearance of IgG directed against both toxins from D21 after infection. Regarding the mucosal response, we observed an anti-TcdA and anti-TcdB IgA response on D69. In contrast, we did not detect any anti-SlpA or anti- FliC serum IgG or colonic IgA regardless the time after infection.

Conclusions: The anti-TcdA and TcdB IgM antibodies appeared as early as the second day after infection and isotype switching led to the appearance of serum IgG and colonic IgA directed against both toxins. In order to understand the lack of IgG-type immune response to SlpA and FliC, different hypothesis can be made. Those antigens could not have been available to the dendritic cells, or not in a sufficient amount. The mice lineage could not be optimal. Or finally, the immune response needs a stress signal, or a by-stander effect.

P3

PROSPECTIVE EVALUATION OF THE ADAPTIVE IMMUNE RESPONSE TO SLPA IN *Clostridium difficile* INFECTION

Assaf Mizrahi[1,2*], Jean-François Bruxelle[1*], Séverine Péchiné[1], Alban Le Monnier[1,2]
* These authors contributed equally to this work

[1] EA 4043 „Unité Bactéries Pathogènes et Santé" (UBaPS), Univ. Paris-Sud, Université Paris-Saclay, 92290 Châtenay-Malabry, France ;[2] Service de Microbiologie clinique et dosage des anti-infectieux, Groupe Hospitalier Paris Saint Joseph, 75014 Paris, France

Clostridium difficile is responsible for various clinical manifestations ranging from mild diarrhea to life-threatening fulminant colitis. Various studies have highlighted the importance of the host immune response to toxins but also colonization factors in *Clostridium difficile* infection (CDI) outcome. Recently, SlpA, the precursor of the S-layer of *Clostridium difficile*, has been evaluated as a target in CDI therapeutics.

We conducted a three-years prospective study in order to evaluate the role of the adaptive immune response to SlpA of patients after a CDI on the outcome. Sera of 87 included patients were collected at the day of CDI diagnosis as well as at an early (5 to 25 days) and late period (25 to 90 days) after infection. A serum was also collected from ten other patients, constituting the control group. We tested on the collected serum the specific total anti-SlpA antibodies as well as the IgM and IgG.

65 patients (75%) presented a single episode, 22 (25%) presented at least one episode of recurrence. We observed a heterogeneous antibody response, mostly for the anti-SlpA specific IgM. During the early and late infection period, we observed a significant elevation of total anti-SlpA antibody levels in patients with a single episode compared to the control group and patients with recurrent CDI. There was no statistical difference in specific anti-SlpA IgM levels between the groups from the day of diagnosis to the late period. Regarding the early and late response against the infection, patients with a single episode had significantly higher anti-SlpA IgG antibody level compared to patients with recurrent CDI.

Three patients presented more than two episodes of recurrence. We observed low levels of specific anti-SlpA IgM and IgG antibody levels with no increase during all the follow-up period (180 days).

To the best of our knowledge, this is the first prospective evaluation of the adaptive immune response to SlpA in patients infected by *Clostridium difficile*.

Our results revealed that SlpA appeared to be immunogenic in patients during the infection. Moreover, patients with at least a recurrence of the infection had significantly less specific anti-SlpA IgG than patients with a single episode.

These results will be helpful for a better understanding of the variability of responses in patients to *Clostridium difficile* infection as well as developing new therapies.

P4

Clostridium difficile INDUCES AN ATP-P2X7 MEDIATED INFLAMMASOME ACIVATION

Ya-Hui Liu[1], Yung-Chi Chang[1], Liang-Kuei Chen[1], Yuan-Pin Hung[2] , Wen-Chien Ko[3] and Pei-Jane Tsai[1]

[1]Department of Medical Laboratory Science and Biotechnology, Medical College, National Cheng Kung University, Tainan, Taiwan; [2]Department of Internal Medicine, Tainan Hospital, Ministry of Health and Welfare, Tainan, Taiwan; [3]Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan

Robust caspase-1-dependent IL-1β production in response to *Clostridium difficile* infection (CDI) suggests the involvement of inflammasome activation. To date, various inflammasome members have been identified, among which NLRP3 and NLRC4 are the most common inflammasomes of the host innate immune system in sensing and reacting to bacterial infection. One of the common features of NLRP3 inflammasome activation in response to diverse stimuli is the signaling pathway that relies on the ATP-P2X7 pathway. Thus, we evaluated the levels of released ATP after CDI. Elevated ATP release in culture supernatants was found after CDI. In accordance with this, treatment with apyrase, an ATP/ADP-hydrolyzing enzyme, diminished *Clostridium difficile*-induced IL-1β releasing. Next, we blocked ATP-sensitive K+ channels in the presence of glyburide or pretreated with A438079, which functions as a P2X7 antagonist to clarify the mechanism by the ATP- P2X7 pathway during CDI. The results showed that IL-1β processing and caspase-1 activation were both significantly impaired by preventing K+ efflux or blocking P2X7. Additionally, the production of IL-1β and IL-18 measured by ELISA were markedly reduced following treatment with these inhibitors. To confirm the dependency of P2X7 in *Clostridium difficile*-mediated inflammasome activation, we performed knockdown experiments in THP-1 cells and monitored the effect of P2X7 depletion on the level of inflammasome activation. These data indicate that the ATP-P2X7 pathway is essential for *Clostridium difficile*-induced inflammasome activation.

P5

PROBIOTICS TO PREVENT INDUCTION OF *Clostridium difficile* INFECTION?

Etifa Perezimor[1], Freeman Jane[2], Wilcox Mark H.[2], Baines Simon D.[1]

[1]University of Hertfordshire, Biological and Environmental Sciences, Hatfield, UK; [2]Leeds Teaching Hospitals NHS Trust and the University of Leeds, Leeds, UK.

Background and aims

Clostridium difficile infection (CDI) remains a healthcare burden and recurrent CDI (rCDI) still affects up to 20-30% of patients. Probiotics are live microorganisms that confer a host health benefit, but evidence of their efficacy in CDI prevention/treatment is controversial. Non-toxigenic *Clostridium difficile* (NTCD) have been used successfully used in animals/humans to reduce CDI incidence. The present study aimed to assess efficacy of two probiotics, Lactobacillus casei Shirota (LcS, Yakult) and a local NTCD, in preventing simulated CDI in an in vitro human gut model.

Methods

CD-negative pooled faeces from healthy volunteers (>65yrs) was used to inoculate the gut models. Two probiotics, LcS (6.2 x 10^9 cfu) and NTCD (1 x 10^8 spores), were dosed into separate gut models prior to CD ribotype 027 spore additions (RT027, 1 x 10^8). Probiotic dosing was for 28d; LcS once-daily and NTCD spores once-weekly. Clindamycin (DA, 33.9 mg/L, QID, 7d) was used to disrupt the gut microflora. Gut model contents were assayed for microflora composition using viable counting techniques and CD cytotoxin production using a Vero cell cytotoxicity assay. Probiotic dosing ceased 14d before the end of the experiment.

Results

LcS dosing resulted in marked increases in lactobacilli and bifidobacterial viable counts. However, during DA dosing these viable counts declined by 4-$\log_{10}$cfu/mL. RT027 spore germination and cell proliferation was observed after DA instillation ceased. Interestingly, another cycle of growth/cytotoxin was observed after LcS dosing ceased. NTCD did not colonise the gut model prior to DA instillation; spores were quiescent and washed out. During DA instillation, NTCD spores germinated and vegetative cells multiplied, whereas, RT027 spores did not germinate and no cytotoxin was produced. NTCD remained vegetative until the end of the experiment and isolated cells retained their non-toxigenic phenotype.

Conclusions

Instillation of NTCD prevented primary CDI in a human gut model, whereas dosing with LcS did not. LcS, if beneficial in antagonism of CD, is unlikely to be due to nutrient/adhesion competition or production of antimicrobials. NTCD may be beneficial not only in treating rCDI but also in the prevention of primary infection. Further work with additional CDI-inciting antimicrobials is needed to better understand the protection that NTCD could confer.

P6

EFFECTS OF INTESTINAL COLONIZATION BY *Clostridium difficile* AND STAPHYLOCOCCUS AUREUS ON MICROBIOTA DIVERSITY IN HEALTHY INDIVIDUALS IN CHINA

Danfeng Dong[1], Qi Ni[1], Chen Wang[1], Lihua Zhang[2], Zhen Li[3], Cen Jiang[1], EnqiangMao[4], Yibing Peng[1]

[1]Department of Laboratory Medicine, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; [2]Department of Laboratory Medicine, Xinhua Hospitial, Shanghai Jiaotong University School of Medicine, Shanghai, China; [3]Department of Laboratory Medicine, Longhua Hospital, Shanghai University of Traditional Medicine, Shanghai, China; [4]Department of Emergency Intensive Care Unit, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.

Background: Intestinal colonization by pathogenic bacteria is a risk factor for infection, and contributes to environmental contamination and disease dissemination. Alteration of gut microbiota also plays a pivotal role in the development of disease. Although *Clostridium difficile* and Staphylococcus aureus are well-recognized pathogens causing nosocomial and community infections, the intestinal colonization was not fully investigated. Herein, we explored their overall carriage rates in healthy adults from the community, and characterized the gut microbiomes of *Clostridium difficile* and S. aureus carriers. Methods: Fecal samples were collected from 1709 healthy volunteers from communities in Shanghai, China, and tested for the presence of *Clostridium difficile*, methicillin-sensitive S. aureus (MSSA), and methicillin-resistant S. aureus (MRSA) using culture-based techniques. To explore differences in the gut microbiome, 16S rRNA gene sequencing was conducted using samples from non-carriers (CH), *Clostridium difficile* carriers (CCD), MRSA carriers (CM), and MSSA carriers (CS). Results: Overall, we detected 12 *Clostridium difficile* and 60 S. aureus isolates, accounting for 0.70% and 3.51% of total isolates, respectively. Eight isolates were determined to be MRSA, accounting for 13.3% of the S. aureus population. Sequencing data revealed that the microbial diversity and richness were similar among the four groups. However, at the phylum level, carriage of *Clostridium difficile* or MRSA was associated with a paucity of Bacteroidetes and an overabundance of Proteobacteria compared with non-carriers. At the genus level, the prevalence of the genera Bacteroides, Prevotella, Faecalibacterium, and Roseburia was decreased in *Clostridium difficile*-positive samples compared with the controls, while the proportion of Clostridium cluster XIVa species was increased. MRSA carriers exhibited a higher proportion of the genera Parasutterella and Klebsiella, but a decreased prevalence of Bacteroides. Compared with MSSA carriers, Klebsiella was the only genus found to be significantly enriched in MRSA carriers. Conclusions: In healthy adults, colonization by *Clostridium difficile* or S. aureus did not significantly affect gut microbiota diversity. However, the alteration of the gut microbiota composition in *Clostridium difficile* carriers could indicate a predisposition to further infection. Our study provides essential data on the prevalence and effects of *Clostridium difficile* and S. aureus colonization on gut microbiota composition in healthy adults.

P7

THE INFLUENCE OF FOOD OLIGOSACCHARIDES (PREBIOTICS) ON ADHESION OF *Clostridium difficile* TO THE HUMAN COLONIC EPITHELIAL CELL LINE (HT29/C1) IN VITRO: A PILOT STUDY

Michał Piotrowski, Dorota Wultańska, Hanna Pituch

Department of Medical Microbiology, Medical University of Warsaw, Warsaw, Poland

Background and aims
Prebiotics are usually short-chain oligosaccharides and are not digested by human digestive enzymes. The function of prebiotics is to provide beneficial effects on the host. Mechanism by which oligosaccharides might to interfere with bacteria is based on the observation that exogenous oligosaccharides may act as molecular receptors that can competitively inhibit bacterial adhesion. The aim of this study was to investigate the effect of food oligosaccharides on the adhesion of *Clostridium difficile* to the Human Colonic Epithelial Cell Line HT29/C1.

Material and Methods
The influence of five oligosaccharides (inulin, fructooligosaccharides (FOS), cellobiose, mannose, and raffinose) on the adhesion of 10 clinical *Clostridium difficile* strains and reference *Clostridium difficile* 630 to HT-29/C1, was investigated. For the experiment, cell line was incubated in 24-well plates with prebiotics and bacteria. Negative control were cells incubated with bacteria, without oligosaccharides. After incubation cells were trypsinized, the contents of all wells were diluted and blood Columbia Agar plates were inoculated by 20µl of dilution in duplicate. Plates were incubated for 48h, at 37oC under anaerobic conditions. The grown colonies were counted. This experiment was performed three times for each strain. Averages and percentage of adhesion were calculated. Data were tested using one-way ANOVA and Tukey's HSD post-hoc analysis.

Results
Most of examined oligosaccharides displayed anti-adhesion properties. Mannose decreased adhesion of *Clostridium difficile* to the colonic epithelial cells to 54,4% ($p<0,001$), FOS and raffinose to 70,2% ($p<0,05$) and 72,6% ($p=0,063$) respectively; cellobiose presented weak anti-adhesion properties (92,5%; $p=0,96$). It is interesting that inulin, often used for the supplementation of diet, increased the adhesion of *Clostridium difficile* to HT29/C1 in vitro (108.1%, $p = 0.97$).

Conclusions
We demonstrated that all prebiotics except inulin, were anti-adhesive for *Clostridium difficile* strains binding to human HT29/C1 cells. Inulin may increase *Clostridium difficile* adhesion, which may have an adverse effect when using for prophylactic purposes in CDI patients with CDI. Further studies evaluating the anti-adhesive properties of food oligosaccharides on *Clostridium difficile* are urgently needed.

This work was supported by the National Science Centre, Poland (Grant number: UMO:2017/25/N/NZ6/01763).

P8

MONITORING BACTERIAL POPULATIONS IN AN IN VITRO MODEL OF THE HUMAN COLON WITH RESOLVED *Clostridium difficile* INFECTION FOLLOWING FAECAL MICROBIOTA TRANSPLANTATION

Moura Ines B.[1], Buckley Anthony M.[1], Normington Charmaine[1], Harris Hannah[1], Ewin Duncan[1], Clark Emma[1], Owen Kate[1], Shearman Sharie[1], Chilton Caroline H.[1], Wilcox Mark H.[1, 2]

Affiliation(s): [1]Healthcare Associated Infections Research Group, University of Leeds, Leeds, UK; [2]Healthcare Associated Infections Research Group, Leeds Teaching Hospitals NHS Trust, Leeds, UK

In recent years, faecal microbiota transplantation (FMT) has been used successfully to treat recurrent *Clostridium difficile* infection (rCDI). In this study, in vitro chemostat models of the human colon were used to evaluate intestinal dysbiosis resulting from antibiotic therapy in rCDI, and the re-establishment of the gut microflora following FMT. Bacterial populations were monitored by standard culture and compared with real-time quantitative PCR (qPCR).

Simulated CDI was induced in three triple stage models (A, B & C) using clindamycin. Model A was subsequently treated with vancomycin, whilst models B and C were treated with vancomycin and a FMT. Targeted bacterial populations were monitored daily using selective agars. DNA extractions were performed in triplicate at key time points. Variations in Bacteroides, Prevotella, Enterobacteriaceae, Clostridium coccoides and Clostridium leptum were monitored by qPCR. Total bacteria were quantified using universal 16S primers and a FAM-tagged probe. Plasmid-based standard curves were used to determine bacterial copy numbers.

According to the qPCR analysis, clindamycin led to significant ($p<0.05$) average declines in Prevotella and Bacteroides (2 logs), and C. coccoides (1 log) groups among all models, whilst Enterobacteriaceae populations significantly increased (2 logs). Vancomycin significantly decreased the levels of all bacterial groups (1-4 logs). A week after cessation of vancomycin, only Enterobacteriaceae populations had started to recover in model A, whereas all populations had increased up to 3 logs in the models that received FMT (B & C). Total bacteria declined 1 log following clindamycin and remained stable throughout the experiment. Bacterial culture also indicated a more severe disruption of the gut microflora following vancomycin instillation, relative to clindamycin. Selective culture showed an average decline in Bacteroides populations of 1.5 $\log_{10}$ cful/ml for clindamycin and 7 $\log_{10}$ cful/ml for vancomycin. rCDI was observed only in model A, 30 days after completion of vancomycin treatment.

Bacterial culture and qPCR data correlated well and together provided a wider perspective on the disruptive effects of antibiotics on the gut microflora. The swift reconstitution of the microbiota in the FMT models successfully prevented rCDI.

USE OF AN IN VITRO GUT MODEL OF RECURRENT CDI TO EVALUATE THE IMPACT OF FAECAL TRANSPLANTS

P9

Charmaine Normington[1], Anthony Buckley[1], Christopher Ford[2], Ines B. Moura[1], Duncan Ewin[1], Sharie Shearman[1], Emma Clark[1], Kate Owen[1], Matthew R. Henn[2], David Cook[2] & Mark H. Wilcox[1]

[1]Molecular Gastroenterology Group, University of Leeds, Leeds, U.K. [2]Seres Therapeutics, Cambridge, MA 02139, U.S.

Background: Antibiotic treatment of *Clostridium difficile* infection (CDI) perpetuates the underlying intestinal dysbiosis, allowing for germination of dormant *Clostridium difficile* spores. Faecal transplants (FMT) from healthy donors have reduced the risk of recurrent CDI (rCDI) in subjects with a history of infections with single-dose efficacy ranging from 67-89%. There is a need for robust model systems to mechanistically probe the impact of FMT on rCDI. In this study, we evaluated the ability of FMT to prevent rCDI using an in vitro triple stage chemostat model of the human colon. Bacterial enumeration and 16SV4 profiling were used to evaluate growth dynamics in both planktonic (free-floating) and sessile (stationary) communities.

Materials/methods: Three triple-stage chemostat gut models were inoculated with faecal slurry. Bacterial populations were allowed to reach steady state before challenge with *Clostridium difficile* spores. Simulated CDI was induced by clindamycin and subsequently treated with vancomycin. Following vancomycin, two models (B&C) were treated with fresh faecal slurry from a single donor (simulating nasogastric FMT). Microbiota and *Clostridium difficile* were monitored from the planktonic and biofilm populations over approximately 100 days. *Clostridium difficile* cytotoxin and antimicrobial concentrations were monitored in the planktonic phase over the same time frame. 16SV4 Illumina sequencing data were generated from a subset of samples.

Results: CDI occurred soon after clindamycin-induced dysbiosis, characterised by *Clostridium difficile* outgrowth and toxin production. Although vancomycin successfully treated CDI in all three models, relapse occurred 20 days post vancomycin in Model A. Simulated FMT therapy (Models B&C) successfully prevented rCDI for the duration of the experiment (four weeks post FMT). 10 days post FMT, microbial populations reached stable levels. Within the biofilm, *Clostridium difficile* was associated with sessile populations, which showed little variation over time.

Conclusions: rCDI was successfully prevented by FMT, which may contribute to colonization resistance. Persistence of *Clostridium difficile* in sessile communities has implications for risk of CDI recurrence in patients undergoing future antimicrobial therapy. Using FMT as a probe, we have also demonstrated the feasibility of using this gut model to evaluate microbiome-based drug therapies to prevent rCDI.

P10

SER-109 PREVENTS RECURRENCE OF *Clostridium difficile* IN A DOSE DEPENDENT MANNER IN AN IN VITRO MODEL OF THE GI MICROBIOME

Anthony M. Buckley[1], Christopher Ford[2], Charmaine Normington[1], Ines B. Moura[1], Duncan Ewin[1], Sharie Shearman[1], Emma Clark[1], Kate Owen[1], Matthew R. Henn[2], David Cook[2] & Mark H. Wilcox[1]

[1]Molecular Gastroenterology Group, University of Leeds, Leeds, U.K. [2]Seres Therapeutics, Cambridge, MA 02139, U.S.

Background: Despite antibiotic treatment, *Clostridium difficile* infection recurs (rCDI) secondary to antibiotic-induced dysbiosis, which is characterized by a relative depletion of Firmicutes and an abundance of Proteobacteria. SER-109, an ecology of bacterial spores purified from stool of healthy donors, is an investigational microbiome therapeutic intended to facilitate microbiome restoration and reduce risk of rCDI. Rapid engraftment of spore-forming species is associated with higher doses of SER-109 in our dose-ranging Phase 1b study of subjects with rCDI. We explored whether higher doses of SER-109 were associated with prevention of rCDI in a triple stage bioreactor model of the human gut.

Methods: Four triple-stage chemostat gut models were inoculated with faecal slurry from a single source. Bacterial populations reached steady state before challenge with *Clostridium difficile* spores and instillation of clindamycin. Primary CDI was treated with vancomycin. Two models were treated with a single dose of research-grade SER-109 (3x108 spore equivalents, SporQ), and two were treated with three doses of research-grade SER-109 over three days (3x108 SporQ each). Commensal microbiota and *Clostridium difficile* were monitored from planktonic and sessile populations over approximately 100 days. *Clostridium difficile* cytotoxin and antimicrobial concentrations were monitored in the planktonic phase over the same time frame. 16SV4 Illumina sequencing data were generated from a subset of samples.

Results: In our model, three doses of SER-109 were able to prevent rCDI for the duration of the experiment (35 days); however, *Clostridium difficile* germination and toxin production was detected following a single dose. Three doses of SER-109 were characterised by a rapid increase in the Clostridium spp. and a reduction in Gammaproteobacteria. *Clostridium difficile* was associated with the sessile populations which did not decrease during either antibiotic or microbial therapy, consistent with a "reservoir" hypothesis.

Conclusions: Higher doses of SER-109 are associated with more rapid recovery of Clostridium spp. and prevention of recurrence in an gut model of CDI. These results are consistent with an in-depth post-hoc analysis of the Phase 1 and Phase 2 SER-109 clinical trial microbiome data. Seres has initiated a Phase 3 study of SER-109 to reduce rCDI, which includes an increase in dose titer and frequency.

P11

DEVELOPMENT OF A PROBIOTIC TREATMENT FOR *Clostridium difficile* INFECTIONS USING HYBRID MICROBES THAT ACT VIA MULTIPLE MODES OF ACTION

Anthony Cann[1], Jeanette Mucha[1], Derik Twomey[1], and Gregory Govoni[1]

[1]SciBac Inc., Burlingame, California, USA.

Background & Aims. New antibacterial therapies are urgently needed for antibiotic-resistant and difficult-to-treat bacteria. One such bacterial species, *Clostridium difficile*, is able to exploit disruptions to the gut microbiota in order to proliferate and cause disease. Due to ineffective treatment options, CDI recurrence rates are upwards of 25%. We propose using our patented gene transfer technology to create hybrid organisms capable of combatting CDI and CDI recurrence. Our first aim is to create hybrid organisms capable of growing in nutrient restricted environments such as the inflamed gut while also demonstrating anti-*Clostridium difficile* properties.

Methods. The DRIVE (Directed Recombination by In Vitro Evolution) gene transfer technology platform harnesses natural horizontal gene transfer to rapidly combine genetic traits from two species into a single hybrid organism. Resulting bacterial hybrids were tested for growth on a carbohydrate panel and minimal media. They were also tested for their effects on 1) *Clostridium difficile* cell viability 2) *Clostridium difficile* adhesion to Caco-2 colon cells and 3) spore viability as measured by CFU. Hybrid yeast strains were tested for anti-toxin activity using an ELISA for *Clostridium difficile* toxins A and B.

Results. The resulting bacterial hybrids were found to be less fastidious than the parent strains. The two most active hybrid bacterial organisms gained the ability to grow on 6 additional carbohydrates and became prototrophic for all amino acid production. These bacterial hybrids were also able to kill all strains tested from a *Clostridium difficile* panel containing common ribotypes. Co-culture of the hybrid organisms with the VPI10463 strain eliminated the strain by 12 hours. In addition, the hybrid organisms fully prevented VPI10463 attachment to Caco-2 colon cells. Finally, multiple hybrid yeast organisms were found to neutralize toxins A and B from VPI10463 by $\geq$ 90%.

Conclusions. Most treatments for CDI are designed to address a single action of *Clostridium difficile* during infection. By creating multiple hybrid organisms via the DRIVE platform, we were able to create a cocktail of hybrid organisms capable of treating multiple modes of action while being better suited to withstand inflamed gut environments. We believe that this multi-factorial approach stands a much better chance for lasting, successful treatment outcomes when it comes to CDI and CDI recurrences.

P12

IMPACT OF SACCHAROMYCES BOULARDII CNCM I-745 ON THE IN VITRO BIOFILM OF *Clostridium difficile*

Bouttier S, Malet J, Mejri Y and Janoir C.

EA4043 Unité Bactéries Pathogènes et Santé (UBaPS), Univ. Paris-Sud, Université Paris- Saclay, 92290 Châtenay-Malabry, France

Background and aims

Clostridium difficile is the leading cause of healthcare-associated diarrhea. Clinical signs range from mild diarrhea to pseudomembranous colitis. Recurrence, which occurs in more than 20% of patients after a first episode of *Clostridium difficile* infection (CDI), can be either due to reinfection with a different strain or to a relapse caused by the initial strain. It is generally recognized that relapses are due to the persistence of *Clostridium difficile* in the form of spores, but bacterial persistence within a biofilm could also be considered. Saccharomyces boulardii CNCM I-745 is a probiotic yeast that can be used, in association with vancomycin, for the treatment of recurrent CDI. This study evaluated the impact of S. boulardii on *Clostridium difficile* biofilm formation.

Methods

Biofilm assays were performed in 24-well polystyrene plates in Brain Heart Infusion broth supplemented with 1.8% D-glucose, 0.1% L-cysteine and 0.5% yeast extract (Difco). Overnight suspensions of *Clostridium difficile* R20291 and of S. boulardii were added to each well after dilution at different ratios. After 72 h of incubation at 37°C under anaerobic conditions, the biofilm biomass was quantified after crystal violet (CV) staining, and viable cells of both *Clostridium difficile* and S. boulardii were enumerated. Biofilm architecture was analyzed by confocal laser scanning microscopy (CLSM). Monospecific biofilm served as control.

Results

S boulardii monospecific culture was shown to form a weak biomass after CV staining. When *Clostridium difficile* and S. boulardii were mixed at 1:1 or 1:10 ratios, respectively, the biomass was more substantial and similar to that of a monospecific *Clostridium difficile* biofilm. In contrast, the number of viable *Clostridium difficile* cells was decreased by at least one log in the mixed biofilm while the number of viable S. boulardii cells was not different from that in the monospecific

S. boulardii biofilm. By CLSM, we showed that the architecture of the mixed biofilm was different from the one formed by *Clostridium difficile* alone, with a significantly reduced thickness and more heterogeneous and alveolate structure.

Conclusion

Our data show that co-incubation of S. boulardii CNCM I-745 with *Clostridium difficile* R20291 results in a modification of the architecture of the *Clostridium difficile* biofilm and of the bacterial viability therein, suggesting the formation of a weaker biofilm by *Clostridium difficile* in presence of the yeast.

P13

Clostridium difficile GROWTH AND CYTOTOXICITY ASSOCIATED BACTERIAL SIGNATURES IN THE IN VITRO MODULATED GUT MICROBIOTA

Aleksander Mahnic[1], Nataša Poklar Ulrih[2], Jennifer Auchtung[3], Robert A. Britton[3], Maja Rupnik[1,4]

[1]National Laboratory for Health, Environment and Food, Maribor, Slovenia; [2]Biotechnical Faculty, University of Ljubljana, Slovenia; [3]Baylor College of Medicine, Houston, Texas, USA; [4]Faculty of Medicine, University of Maribor, Slovenia

We have used MBRA (mini-bioreactor arrays) model to study bacterial signatures associated with *Clostridium difficile* growth and cytotoxicity after in vitro modulation of fecal microbiota.

Clindamycin and polyphenol extracts from pomegranate and blueberries were used alone and in combinations as microbiota modulating factors in the MBRA gut model. After stabilization of modulated microbiota we inoculated bioreactors with *Clostridium difficile* vegetative cells (ribotype 027) followed by a 7-day periodical monitoring of *Clostridium difficile* growth, activity of toxin A and B (cytotoxicity assay with HT-29 and Vero cell line, respectively) and bacterial community composition (16S metagenomics targeting V3-V4 hypervariable region of the bacterial 16S rRNA gene).

Modulation with clindamycin and/or polyphenol extract resulted in unique microbial communities. However, all conditions resulted in a successful overgrowth of *Clostridium difficile*, while cytotoxicity varied significantly among conditions. Only in a single condition the cytotoxicity was decreased despite successful *Clostridium difficile* growth. Using 16S metagenomic data we were able to correlate three bacterial taxons (C. sporogenes, C. oroticum and Blautia sp.) with decreased cytotoxicity. Representatives were selectively isolated from bioreactor slurry and used for co-cultures with *Clostridium difficile*. We showed decreased cytotoxicity in the presence of C. sporogenes while *Clostridium difficile* growth was not largely affected.

In an in vitro gut model both clindamycin and/or polyphenols modulate fecal microbial community toward the loss of resistance against *Clostridium difficile* colonization. Modulation with clindamycin resulted in a higher *Clostridium difficile* cell concentration and cytotoxicity with an exception of treatment with pomegranate extract as modulating factor. We show that among three differentially represented bacterial taxa coinciding with this trend only C. sporogenes shows the ability to reproduce comparable effect in an in vitro co-culture model with *Clostridium difficile*.

P14

ISOGENIC BINARY TOXIN *Clostridium difficile* MUTANT (CDT-) SHOWS DECREASED ADHERENCE IN VITRO COMPARED TO THE PARENT (CDT+) STRAIN

Farida Siddiqui[1,5], Jay Radke[2], Adam Cheknis[1], Chen Chen[3], Joseph Barbieri[3], Lorinda Guenther-Wright[1], Bob Carman[4], Matt Lyerly[4], Dale Gerding[1], Stuart Johnson[1,5]

[1]Hines VA Hospital, Hines, IL, U.S., [2]Boise VA Hospital, Boise, ID, U.S., [3]Medical College of Wisconsin, Milwaukee, WI, U.S., [4]Tech Lab, Blacksburg, VA, U.S., [5]Loyola University Medical Center, Maywood, IL, U.S.

CDT (*Clostridium difficile* transferase) is a binary, actin ADP-ribosylating toxin frequently associated with hypervirulent *Clostridium difficile* strains. This toxin is encoded by the CDT locus (CdtLoc), composed of cdtA and cdtB. CDTa is the enzymatically active component, whereas CDTb is the binding component. Together CDTa and CDTb lead to the disassembly of the actin cytoskeleton, and eventually to cell death. Adherence to intestinal epithelial cells is the most probable initial step in the pathogenesis of *Clostridium difficile* infection (CDI). It has been proposed that binary toxin CDT may enhance bacterial adherence and colonization by inducing the formation of microtubule-based protrusions on the surface of epithelial cells exposed to CDT.

To determine the effect of CDT on *Clostridium difficile* adherence in vitro, we first constructed a cdtA-null mutant of the toxin A+/toxin B+/CDT+ *Clostridium difficile* strain, BK12 using Clostron technology. The A+/B+/CDT- BK12 mutant was analyzed using PCR to demonstrate intron integration and to confirm the location of the insert in the target gene. Functional ADP ribosylation activity was quantified by measuring incorporation of biotinylated NAD+ into HeLa cell lysate with exogenous actin, and was found to be completely abrogated in the mutant. Expression of the binding component of binary toxin (CDTb) was measured in 48h cultures by ELISA and was reduced 2.5-fold in the mutant compared to the wild type. Some expression of CDTb remained likely due to the intact cdtB gene in the mutant. We then compared in vitro adherence using the colonic epithelial cell line, SKCO-15. After 2h in an anaerobic chamber, planktonic *Clostridium difficile* were removed and cfu/ml of adherent bacteria was calculated by spreading serial dilutions on TFA plates and counting resulting colonies. Percent adherence was determined for the BK12 wild type (CDT+) and BK12 mutant (CDT-) strains and with other well-characterized CD strains; BI, AA1p and non-toxigenic M3 for comparison. Adherence varied greatly between strains with different genotypic backgrounds. However, the CDT- BK12 mutant exhibited significantly decreased adherence (12%) compared to the wild type CDT+ BK12 strain (28%).

Disruption of CDTa and abrogation of ADP ribosylation activity results in decreased epithelial cell adherence in vitro, and provides additional support for CDT as a virulence factor for *Clostridium difficile*.

P15

CLARIFYING THE MECHANISMS OF RIBOTYPE 002 VIRULENCE IN *Clostridium difficile* INFECTION

Ka Yi Kong[1], Thomas Kwong[1], Margaret Ip[2], Raphael Chan[2], Lin Zhang[3], William Wu[3], Sunny H Wong[1]

[1] Institute of Digestive Disease, Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong
[2] Department of Microbiology, Faculty of Medicine, The Chinese University of Hong Kong
[3] Department of Anaesthesia and Intensive Care, Faculty of Medicine, The Chinese University of Hong Kong

Background and aim: Clostridium difficile (*Clostridium difficile*) is a spore-forming, gram-positive anaerobic bacterium that causes diarrhea and pseudo-membranous colitis. We previously reported that PCR ribotype 002 is a prevalent ribotype in Hong Kong, and was associated with a higher mortality. The project aims to investigate the virulence mechanisms of common ribotypes.

Methods: Clostridium difficile was cultured in Brain-Heart Infusion (BHI) medium and incubated in an anaerobic chamber. Beside ribotype 002, ribotype 012, 014, 046 and a hypervirulent ribotype 027 were chosen. Growth rate was determined by measuring the optical density (OD600) at different timepoints, i.e. 0h, 4h, 8h, 24h, 48h. Cell cytotoxicity and neutralization assay (CCNA) was performed to check for toxin B presence. ELISA was done to determine the concentrations of toxin A and B in the supernatant of the culture collected and filtered at 24h, 48h and 72h.

Sporulation and germination experiment was performed using the sample from liquid culture at 0h, 24h, 48h and 72h and heated for 25min at 60°C and an unheated sample as control. The samples were transferred to agar plates. Colony forming unit (CFU) was counted after 24h. Sporulation frequency was calculated by dividing CFU of heated sample over CFU of unheated sample on BHI agar with 0.1% taurocholate. Germination frequency was calculated by dividing the CFU of heated sample on BHI agar without taurocholate over CFU of heated sample on BHI agar with taurocholate.

Results: Ribotype 002 had a longer stationary phase in the growth curve than other ribotypes. CCNA confirmed all strains had the presence of toxin B. Ribotype 002 showed high concentrations of toxin A and B than ribotype 012 and 046 (In toxin A 002: 88.27ng/ml vs 012: 35.05ng/ml, $p=0.001$; In toxin B 002: 74.75ng/ml vs 046: 1.846ng/ml, $p<0.0001$). Meanwhile, 002 had a significantly higher sporulation rate than 014 and 046 at 72h (002: 47.14% vs 014: 7.341%, $p<0.0001$; 002: 47.14% vs 046: 11.64%, $p<0.0001$). Besides, 002 showed a significant higher germination rate that other ribotypes at 0h and 48h (At 0h, only 002 showed 5.615%, $p<0.02$ compare to other groups with 0%; at 48h, 002 showed 7.687% vs 012: 0.5144%, $p<0.0001$).

Conclusions: Ribotypes 002 was shown to have high toxin production, sporulation and germination rate, which may contribute to the high mortality of *Clostridium difficile* riboytpe 002 in Hong Kong.

P16

PHENOTYPIC CHARACTERIZATION OF NON-TOXIGENIC *Clostridium difficile* ISOLATED FROM PATIENTS IN MEXICO.

Sanchez –Rojas M[1]., Camorlinga-Ponce M[2]., and Torres J[2]., Romo-Castillo M[3].*

*[1] Facultad de Ciencia y Tecnología, Universidad Simón Bolívar, Ciudad de México 03920, México. [2] Unidad de Investigación en Enfermedades Infecciosas, Hospital de Pediatría, Centro Médico Nacional-Siglo XXI, Instituto Mexicano del Seguro Social, Ciudad de México 06720, México. [3] CONACYT-IMSS-Unidad de Investigación en Enfermedades Infecciosas, Centro Médico Nacional-Siglo XXI, Instituto Mexicano del Seguro Social, Ciudad de México 06720, México. *email: mariana.romo@live.com.mx*

Clostridium difficile is a Gram positive, anaerobic bacteria that sporulated to survive in aerobic conditions. This bacteria has been reported as the first cause of nosocomial diarrhoea, ranging from 10% to 35% of the diarrhoea cases associated to the excessive use of antibiotics. *Clostridium difficile* was isolated from human faeces in an asymptomatic patient and then considered part of the intestinal microbiota, until 1970 when it was recognized as the causal agent of pseudomembranous colitis. Recently, the incidence and severity of *Clostridium difficile* infection (CDI) are increasing as a result of the emergence of new hypertoxigenic strains, which have new virulence factors. There are two principal virulence factor needed for CDI, the enterotoxin TcdA, and the cytotoxin TcdB. However there are some strains that are unable to produce this toxins, and are denominated Non-toxigenic *Clostridium difficile* (NTCD).The evolutionary history of NTCD has not been cleared, and the relation of these strains with illness remains in doubt. So, the aim of this work is to analyze the phenotype of NTCD strains isolated from clinical cases from a hospital in Mexico, and analyze whether the heterogenicity of these strains, may be useful to differentiate NTCD strains from the toxigenic strains. We analyzed phenotypic characteristics like sporulation, motility, adherence and colony morphology to compare NTCD strains with Toxigenic strains. Our preliminary results indicates that sporulation and colony morphology are different in NTCD strains.

P17

PLEOTROPIC REGULATORY PROTEINS SINR AND CD2215 ARE ASSOCIATED WITH BIOFILM FORMATION, MOTILITY AND SPORULATION IN *Clostridium difficile*

Helen A. Shaw[1], Alexandra Faulds-Pain[1], Mark D. Preston[1], Irene Bianconi[2], Fauzy Nasher[1], Hong Huynh[2], Neil Fairweather[3], Simon Cutting[2], Brendan W. Wren[1] and Lisa F. Dawson[1]

[1]Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK; [2] School of Biological Sciences, Biomedical Sciences, Royal Holloway, University of London, Egham, Surrey, TW20 0EX; [3] Faculty of Natural sciences, Department of Life Science, Imperial College London, Flowers Building, South Kensington Campus.

Clostridium difficile is the leading cause of nosocomial diarrhoea worldwide, ranging from mild diarrhoea to the life threatening pseudomembranous colitis. *Clostridium difficile* infection (CDI) is linked to antibiotic treatment, which disrupts the natural gut microbiota and enables *Clostridium difficile* to proliferate. Relapse rates of CDI are high, yet little is known about colonisation of the gut and adaptation of *Clostridium difficile* to its niche. Understanding the mechanisms behind colonisation, relapse and virulence in *Clostridium difficile* will provide an insight into development of novel treatments for *Clostridium difficile*. Herein we describe the role of the pleotropic transcriptional regulators SinR (CD2214) and CD2215. In B. subtilis SinR is a known transcriptional repressor, which along with its aganoist SinI regulated biofilm formation, under the control of Spo0A, the sporulation master regulator. We show that *Clostridium difficile* contains an orthology of SinR (CD2214), located upstream of CD2215. Unlike SinI in B. subtilis, CD2215 is a putative DNA binding protein with a HTH motif. We have assessed the role and function of these two DNA binding proteins in *Clostridium difficile* by creating insertional inactivation mutants in SinR and CD2215. Using transcriptional profiling, we determined that SinR and CD2215 modulate expression of a wide variety of genes, including cell wall proteins, flagella and putative sortase substrates, and may be involved in the sporulation and biofilm pathways. Electromobility shift assays have shown that CD2215 binds within the promoter upstream of SinR to regulate transcription therein. We assessed the role of these regulatory proteins in-vitro and in-vivo to assertain their function. Our findings highlight an important attribute of *Clostridium difficile*, which may have significant implications for *Clostridium difficile* infection, treatment and relapse.

P18

PUTATIVE VIRULENCE FACTORS IDENTIFIED IN LARGE CLOSTRIDIAL NEGATIVE, BINARY TOXIN PRODUCING *Clostridium difficile* STRAINS

Androga Grace[1,2], Knight Daniel[3], Hutton Melanie[4], Chang Barbara[1], Foster Niki[5], Lyras Dena[4], Riley Thomas[1, 2, 3, 6]

[1]The University of Western Australia, School of Biomedical Sciences, Western Australia, Australia; [2]PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Western Australia, Australia; [3] Murdoch University, School of Veterinary and Life Sciences, Western Australia, Australia; [4] Monash University, Department of Microbiology, Victoria, Australia; [5] Department of Health, Communicable Disease Control Directorate, Western Australia, Australia; [6] Edith Cowan University, School of Medical and Health Sciences, Western Australia, Australia

Background: The relevance of binary toxin [CDT] production by large clostridial toxin negative [A-B-CDT+] *Clostridium difficile* strains is unknown. These strains are considered less likely to cause disease but are currently raising concerns about potential pathogenicity due to their isolation from symptomatic individuals. We investigated putative virulence traits in A-B-CDT+ *Clostridium difficile* strains that may contribute to their role in idiopathic diarrhoea.

Methods: Phenotypic assays were conducted on 148 A-B-CDT+ strains of *Clostridium difficile* comprising 10 different ribotypes (RTs), 53 of which were whole genome sequenced to identify genetic elements associated with virulence and survival.

Results: Analysis of the strains indicated differences in phenotype and genotype relating to motility, toxicity, adherence and immunogenecity. Of the 148 strains, 66% were non-motile [all RT033 and RT288] and had deletions in F2 [glycosylation genes] and F3 [early-stage flagellar genes] regions of their flagellar operon. Seven RTs were slightly motile but significantly less motile than the reference strain R20291 and lacked the glycosyltransferase genes essential for *Clostridium difficile* flagellum assembly and full motility trait. The flagellin and flagella cap genes, fliC and fliD, involved in adherence and host colonisation were conserved in all strains including reference strains. In silico proteonomic analysis identified putative adhesin encoding genes, cwp2, cwp66, cwp84, secA2, slpA, fbp68 and cbpA, that also act as immunogenetic factors and play a role in bacterial attachement and pathogenesis of infection. All isolates produced at least 3 extracellular enzymes [deoxyribonuclease, esterase, mucinase]. We confirmed toxicity of the strains in Vero cells, however, this was not demonstrated in a mouse model of disease. Mice infected with A-B-CDT+ *Clostridium difficile* strains all survived infection despite detection of high numbers of spores [107 CFU/g] in the faeces at either 24h or 96h post-infection. None had diarrhoea with the exception of mice infected with strain QX146. These mice had soft faeces/diarrhoea 24h post-infection and showed weight loss, however, went on to recover from the infection.

Conclusion: This study provides the first in-depth analysis of A-B-CDT+ *Clostridium difficile* strains and highlights the need to further investigate their role in C.difficile disease.

PHENOTYPIC CHARACTERISATION OF AN EMERGING MLST CLADE 2 LINEAGE OF *Clostridium difficile*, RIBOTYPE 251

P19

Hong Stacey [1], Knight Daniel R [2], Chang Barbara [1], Carman Robert [3], Riley Thomas V. [1, 2, 4, 5]

[1]*School of Biomedical Sciences, Faculty of Health and Medical Sciences, The University of Western Australia, Crawley, Western Australia, Australia;* [2]*School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, Australia;* [3]*TechLab Inc., 2001 Kraft Drive, Blacksburg, Virginia 24060 – 6358, United States of America;* [4]*PathWest Laboratory Medicine, Department of Microbiology, Queen Elizabeth II Medical Centre, Nedlands, Western Australia, Australia;* [5]*School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia.*

Background and aims: The binary toxin positive strain of *Clostridium difficile* ribotype (RT) 027 caused major outbreaks in North America and remains highly prevalent in the USA. Although RT027 has never established in Australia, a genetically related strain, RT251, has increased in prevalence Australia-wide since 2010. Herein, we phenotypically characterised a selection of RT251 strains to ascertain virulence and significance in human infection.

Methods: Clostridium difficile spore germination and outgrowth were monitored at OD600. The rate of sporulation was determined using differential spore-plating CFU measurements. Toxin A and toxin B production were quantified using tgcBIOMICS and TechLab ELISA kits. In vitro toxin production was confirmed by Vero cell and HT-29 cytotoxicity assays. Motility assays (0.175% BHIA) were performed and antimicrobial susceptibility was determined using agar incorporation methods.

Results: Clostridium difficile RT251 strains showed rapid germination (≈5 min) compared to a RT027 strain (R20291; ≈20 min). Highest spore outgrowth was observed for a RT251 strain (unpaired t-test, P=0.05, 12h) compared to VPI10463. In contrast, VPI10463 showed better growth in standard growth curve assays, reaching stationary phase earlier than other strains (11h vs 14.7h). The sporulation rate was highest at 48h for all strains, except for an unusual RT251 strain where no spores were seen by 120h. Toxin filtrates induced typical cytopathic effect (CPE) on Vero and HT-29 cells. Toxin production by all RT251 strains of *Clostridium difficile* was comparatively lower than strains VPI10463, 630 and R20291 in cell culture and ELISA (TechLab). Toxin A and B were quantified separately using tgcBIOMICS ELISA (detectable range: 0.31–40 ng/mL). Toxin A plateaued by 48h for VPI10463 and 630. However, both R20291 and RT251 strains demonstrated robust toxin A and B production, with highest levels at 24h and 72h, respectively. All RT251 isolates were motile and susceptible to metronidazole and vancomycin; one showed clindamycin and erythromycin resistance.

Conclusions: Clostridium difficile RT251 strains produced spores that germinated faster than those of an epidemic RT027 strain. Despite lower toxin production, RT251 strains produce cytotoxin that induced significant CPE in cell culture. Further studies are required to highlight the importance of RT251 strains in human infection.

P20

STRUCTURAL CHARACTERISATION OF THE *Clostridium difficile* S-LAYER

Paola Lanzoni[1], Anna Barwinska-Sendra[1], Oishik Banerji[2], Arnaud Baslé[1], Per A. Bullough[2], Robert P. Fagan[2], Paula S. Salgado[1]

[1]Institute of Cell and Molecular Biosciences, Faculty of Medical Sciences, University of Newcastle, Newcastle upon Tyne, NE2 4HH, UK, United Kingdom
[2]Krebs Institute and Dept. of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK

Clostridium difficile expresses a surface layer (S-layer) which coats the surface of the bacterium and is proposed to facilitate interactions with host enteric cells. In C. difficile, the S-layer is composed of two SLPs which are derived from post-translational cleavage of a single precursor, SlpA. The SlpA pre-protein contains a signal peptide directing translocation across the cell membrane, after which cleavage occurs producing the mature SLPs: a high-molecular-weight protein (~40 kDa, HMW SLP) and a low-molecular-weight protein (~35kDa, LMW SLP). These proteins form a tightly associated non-covalent complex, the H/L complex, and the regions of both proteins responsible for complex formation have been identified (Fagan et al., 2009). Although the structure of the LMW SLP has been determined (Fagan et al., 2009), structure of the interacting complex or the HMW SLP have remained elusive.

Here we will report our structural efforts using a combination of X-ray crystallography and 2D electron diffraction to allow the structural determination of these interacting regions of the SlpA proteins and to address organisation of S-layer assembly. We have obtained diffracting crystals of the whole LMW/HMW complex from different *Clostridium difficile* strains and solved the structure of ribotype 017 SlpA using S-SAD data from I23, Diamond Light Source, combined with related models and crystal averaging. Together with our recently determined structure of the native *Clostridium difficile* S-layer to 20 Å using electron crystallography, this structural information will allow us to develop a model for the assembly and structure of the mature S-layer in *Clostridium difficile*.

P21

Clostridium difficile TOXIN A INHIBITES WNT / β-CATENIN PATHWAY IN VIVO AND IN VITRO VIA RAC-1 INACTIVATION

Conceição da Silva Martins[1], José Garcia Ribeiro Abreu Júnior[2], Gerly Anne de Castro Brito[1]

[1]Nucleos of Studies in Microscopy and Image Processing; Federal University of Ceara, Faculty of Medicine; [2]Laboratory of Vertebrate Embryology; Federal University of Rio de Janeiro

The objective of this study was to evaluate the changes induced by toxina A of the *Clostridium difficile* (TcdA) in the Wnt/β-catenin pathway in mice and to investigate the role of Rac-1 in the inhibitory activity of this toxin in the Wnt/β-catenin pathway in vitro. For this, C57BL/6 mice were submitted to the ileitis model, through the injection of TcdA (10 µg / loop) or PBS (200 µl; control) on the ileal loop, 4 hours later the animals were euthanized, and segments of the ileum were collected to perform the histopathological analysis and to evaluate changes in Wnt/ β-catenin pathway components by immunohistochemistry (Wnt-3a, β-catenin, cyclin D1, Ki67) and qPCR (cMYC, cyclin D1 and Wnt-3a). Mouse intestinal epithelial cells (IEC-6) were transfected with pcDNA3-EGFP-Rac1-Q61L or pcDNA3 and incubated with TcdA (50 ng/ml) in the presence or absence of Wnt-3a conditioned medium. After 24h incubation, the activation of the Wnt/β-catenin pathway (through the TOP/FOPflash luciferase assay), nuclear β-catenin translocation by immunofluorescence and cell proliferation (immunocytochemistry for Ki67) was evaluated. In vivo, TcdA increased Wnt-3a Immunohistochemical staining and decrease β-catenin and cyclin D1 number of positive cells imunostaining, TcdA significantly decreases gene expression of β-catenin, cMYC, and Ciclin D1, increasing, on the other hand, the Wnt-3a gene expression. TcdA did not alter Rac 1 gene expression in the ileum tissue. In addition, TcdA decreased cell proliferation which has been demonstrated by reducing the number of Ki67 positive cells in the intestinal crypts. In intestinal epithelial cells (IEC-6), TcdA decreased the activation of the Wnt-β-catenin pathway by the pathway agonist (Wnt-3a), reducing β-catenin translocation to the nucleus and decreased proliferation, while transfection of IEC-6 with pcDNA3-EGFP-Rac1-Q61L reversed the TcdA effects in the presence of Wnt-3a. Thus, our results suggest that TcdA inhibits the Wnt/β-catenin pathway in vivo. In addition, the in vitro results show a key role of Rac1 in TcdA induced inhibition of Wnt/β-catenin pathway.

P22

EFFECTS OF *Clostridium difficile* -TOXIN A AND B ON ENTERIC GLIAL CELLS

Costa Deiziane VS[1,2]; Moura-Neto Vivaldo[3] ; Bolick David T[2] ; Nafziger Andrew JH[2]; Medeiros Pedro HQS[1]; Ledwaba SE[2]; Silva Angeline MHP[1]; Martins CS[1]; Guerrant Richard L[2] ; Warren Cirle A[2]; Brito Gerly AC[1*]*

[1]Federal University of Ceara, Department of Morphology, Faculty of Medicine, Ceara, Brazil ; [2]University of Viginia, Department of Infectious diseases, Charlottesville,VA, United States ; [3]Paulo Niemeyer Brain Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.
**equal contribution.*

Background and aims: Increased risk of functional diarrhea has been reported in patients after *Clostridium difficile* infection (CDI). Enteric glial cells (EGCs) regulate neuron function and contribute to inflammatory response. We investigated the effects TcdA and TcdB on the S100B/RAGE/NFκB pathway in ECGs and their role in the pathogenesis of CDI.

Methods: Rat EGC line CRL2690 (ATCC) was incubated with TcdA or TcdB at various doses for 30 min to 48h to evaluate cell viability through MTT assay and cell morphology. Gene expression of glial factors (GDNF, GFAP and S100 β), RAGE receptor (RAGE) and proinflammatory cytokines (IL-1β and IL-6) were performed by qPCR. The levels of S100B were evaluated by Western Blot and ELISA. NFκB activation was evaluated by immunofluorescence. Expression of glial factors was also examined in TcdA-challenged ileal tissues and cecal tissues of VPI10463-infected mice.

Results: TcdB (1, 5 and 10 ng/mL) and TcdA (50 and 100 ng/mL) decreased EGCs viability after 12h and 24h incubation and induced EGC rounding after 30min and 6h, respectively. Similarly, TcdB induced earlier expression of S100B and GDNF compared to TcdA. However, for GFAP there was an initial decrease in transcript expression before increasing at at later timepoint after incubation with either TcdA or TcdB. Both TcdA and TcdB, at all doses used, increased IL-6 transcripts after 12h but only TcdB (1 and 10 ng/mL) increased IL-1β transcripts after 18h. Both TcdA (50 ng/mL) and TcdB (1 ng/mL) stimulated NFκB activation and RAGE expression by EGCs. S100 β, GFAP, GDNF and RAGE expressions were increased in mouse ileum treated with TcdA and cecal tissues from *Clostridium difficile*-infected mice.

Conclusion: TcdA and TcdB induce EGCs morphological changes, loss of viability, and release of S100B, cause NFκB activation and alters expression of glial factors (GFAP and GDNF), RAGE and IL-6 in vitro and in vivo. These findings suggest the involvement of EGC in *Clostridium difficile* pathogenesis.

P23

IMPACT OF THE HIGHLY PREVALENT PHI027 PROPHAGE ON THE BIOLOGY AND VIRULENCE OF THE EPIDEMIC *Clostridioides difficile* STRAIN RIBOTYPE 027.

Garneau R. Julian[1,2], Turcotte Annie[2], Monot Marc[1,2,3], Valiquette Louis, Fortier Louis-Charles[2]

[1]Laboratoire Pathogenèse des Bactéries Anaérobies, Institut Pasteur, Paris, France; [2]Département de Microbiologie et d'infectiologie, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, Sherbrooke, QC, Canada ; [3]Université Paris Diderot, Sorbonne Paris Cité, Paris, France.

Background and aims: Nearly every strain of *Clostridioides difficile* harbors one or more prophages in its genome, however, the information related to their contribution in this species is still very scarce. Of note, the highly prevalent ribotype 027 (R027) strains, responsible for major outbreaks and long considered as hypervirulent, carry a variety of prophages including the ubiquitous phi027. The R027 strains have long been associated with higher mortality rates, severe disease symptoms and greater risk of relapse. Yet, additional investigations revealed that R027 are not always associated with such symptoms and that virulence phenotypes are more variable than previously alleged. These opposing studies highlight the importance to further study the genetic and phenotypic variations in this clinically important strain. Thus, since phi027 prophage represents a significant fraction of each R027 genome, and since our preliminary analyses revealed the existence of numerous phi027 variants, we sought to unveil the role of this omnipresent prophage on the biology of the R027 strain.

Methods: Whole genomes of 70 R027 strains were sequenced using Illumina. Prophage regions were detected using available softwares (Phaster) and further induced with mitomycin C. A bioinformatic approach using *Clostridium difficile* genome database was implemented to identify R027 strains potentially sensitive to phi027, and the predicted sensitivity was confirmed by plaque assays. Phages were purified and a common R027 host was used to create a series of lysogens with the phi027 variants. Using lysogens and wild-type strains, we initiated the comparative evaluation of different parameters, including growth kinetics and sporulation rates.

Results and conclusion: Bioinformatic analyses revealed a total of 390 prophage-like regions (complete and incomplete) within R027 genomes, which could be further categorized in 12 groups based on sequence identity. Analysis of the phi027 regions revealed their presence in every strain and we could distinguish 33 phi027 genomes with at least one minor genetic variation (SNPs, insertions, deletions). Phi027 prophages with the most appreciable variations were selected for further investigations. A total of 6 phi027-like phages, including the phi027 from the epidemic R20291 strain, were amplified for the creation of lysogens used in an array of phenotypic experiments.

P24

Clostridium difficile PEPTIDOGLYCAN REMODELLING DURING ENGULFMENT

Abigail Kelly[1], Marcin Dembek[1], Waldemar Vollmer[1,2], Joe Grey[3] and Paula S Salgado[1].

[1]Institute for Cell and Molecular Biosciences, Newcastle University, England. [2] The Centre for Bacterial Cell Biology, Newcastle University, England. [3]Pinnacle, Newcastle University, England.

Spores are the infective agent in *Clostridium difficile* infections, making sporulation an important, yet unexplored potential therapeutic target. Recent studies have already revealed some differences in sporulation to the model organism B. subtilis, highlighting the need to understand the molecular details involved in *Clostridium difficile*.

Sporulation involves extensive remodeling of the mother cell peptidoglycan during engulfment of the forespore and production of the mature spore. The molecular details of this remodeling and the specific alterations involved are the topics of our current work.
Through a combination of RP-HPLC, LC-MS and mutagenesis, we are investigating the alternations to the fine architecture of peptidoglycan throughout sporulation.

Clostridium difficile vegetative cell and spore peptidoglycan will be characterised as endpoints, whilst mutants that are unable to complete engulfment will demonstrate the dynamic process of peptidoglycan remodelling during sporulation. The catalytic activity of the DMP machinery (formed of SpoIID, SpoIIM and SpoIIP) is also being investigated through the use of catalytic and structural mutant proteins and LC-MS.

A thorough understanding of the fine architecture of *Clostridium difficile* peptidoglycan, coupled with a more detailed knowledge of the mechanics of forespore engulfment will aid the discovery of novel *Clostridium difficile* infection control methods.

DEVELOPMENT OF AN IN VITRO TEST OF COLONISATION RESISTANCE TO *Clostridium difficile* IN FAECAL SAMPLES

P25

Harris Hannah[1], Best Emma[1], Wilcox Mark[1], Chilton Caroline[1]

[1]Healthcare Associated Infection (HCAI) Research Group, Leeds Teaching Hospitals NHS trust and University of Leeds, Leeds, United Kingdom, LS1 3EX.

Background: Investigating factors that can influence colonisation resistance to *Clostridium difficile* infection (CDI) is difficult to achieve using current in vitro techniques. We aimed to develop an assay that could be used to determine colonisation resistance of faecal samples, to facilitate investigation of parameters that may affect host risk of CDI.

Methods: We modified a method first described by Borriello and Barclay (1986). Faecal samples from healthy donors were emulsified with pre-reduced PBS to create slurries. For each test, slurries (20 mL) were prepared in both the raw (untreated) and autoclaved forms, and inoculated with *Clostridium difficile* 027 210 spores (5 log cfu/ml). *Clostridium difficile* total viable counts (TVC) and spores were enumerated at 0, 48 and 72 hours by culture on CCEYL. Cytotoxin production at 72 hours was assessed using the Vero cell cytotoxicity assay. To optimise the assay, different slurry dilutions (1:40 to 1:5 in 2 fold dilutions), storage conditions (+4oC, -80oC, room temperature, -80oC with glycerol, and -80oC with multiple freeze thaw cycles) and sterilisation methods (autoclaving 121 oC for 15 mins, and filtration through 0.2 µM filter) were assessed.

Results: No signs of *Clostridium difficile* spore germination, proliferation or cytotoxin production after 72 hours were observed in raw faecal slurries. However, increases in *Clostridium difficile* populations and cytotoxin production were evident in both autoclaved and filtered samples (~3.5 log10 cfu/ml, 4 RU increase). Effects on colonisation resistance were most apparent with the 1:10 dilution of samples. Storage temperature and freeze thaw cycles had limited effects, but storage with 30% glycerol inhibited *Clostridium difficile* growth and cytotoxin production under all conditions tested.

Conclusion: We have developed a modified in vitro method that reproducibly assesses colonisation resistance to *Clostridium difficile* using volunteer faecal samples. Colonisation resistance was maintained in untreated faecal samples, but not in those that were filtered or autoclaved. This is a robust assay, which was not affected by sample storage temperature, but was affected by the presence of 30% glycerol. The assay is suitable for the assessment of colonisation resistance in clinical faecal samples.

P26

CHEMOTAXIS IN *Clostridioides difficile*

Julian Schwanbeck, Ines Oehmig, Andreas Zautner, Ortrud Zimmermann, Uwe Groß, and Wolfgang Bohne

University Medical Center Göttingen, Department for Medical Microbiology

Most *Clostridium difficile* genomes contain in addition to flagellar genes a genomic region that encodes for a complete chemosensory system. Although a chemotactic effect is not described in *Clostridium difficile* so far, we postulate that chemotaxis might also be involved in this organism in finding niches with optimal growth conditions. One of the genes within the chemosensory operon encodes for a methyl-accepting chemotaxis protein (MCP), a chemoreceptor that possesses a sensory unit and a trans-membrane signal transducer domain. In our ongoing studies we attempt to elucidate the precise role of the C. difficile MCP for chemotaxis and seek to unravel its substrate specificity and its interaction with other components of the chemotaxis system. For this purpose, we generated a MCP knock-out mutant in *Clostridium difficile* 630Δerm by Clostron mutagenesis and are currently investigating its phenotype (Heap, 2010). In order to elucidate which exact chemical stimuli are sensed by C. difficile we are in the process to establish a robust chemotaxis assay. For this purpose we are comparing various chemotactic assays for providing reliable results with *Clostridium difficile*, including soft agar based long-term assays, chemical in-plug assays and short-term capillary assays. To establish a capillary based chemotaxis assay, we tweaked an earlier design (Adler, 1973) to our needs. Bacteria are loaded in capillaries with an inner diameter of 0.35 mm and are incubated for an hour in prospective attractants. Ligands to be tested include sugars, amino acids, metabolites and bile acids. Taken together, this project will contribute to a better understanding of chemotactic processes for *Clostridium difficile* pathogenesis, particularly since motility and toxin production are closely linked in this organism.

References:
Heap et al.: The ClosTron: Mutagenesis in Clostridium refined and streamlined. Journal of Microbiological Methods (2010), 80(1), 49-55.
Adler: A Method for Measuring Chemotaxis and Use of the Method to Determine Optimum Conditions for Chemotaxis by Escherichia coli. Journal of General Microbiology (1973), 74, 77-91.

Acknowledgment
This work was funded by the Federal State of Lower Saxony, Niedersächsisches Vorab (VWZN2889/3215/3266.)

P27

INVESTIGATING THE BIOLOGICAL CONTRIBUTION OF *Clostridium difficile* TYPE IV PILI PROTEINS

Ziyi Wang[1], *Anna Barwinska-Sendra*[1], *Paula S. Salgado*[1]

[1]*Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, UK*

Clostridium difficile has been identified as a multidrug resistant intestinal pathogen, which causes a health concerns in hospital environments. Adhesion, colonization and biofilm formation play important roles in *Clostridium difficile* pathogenesis. It has been indicated that Type IV pili (TFP) contributes to those invading strategies and further estimated that it may even work as a sensor to direct the bacterial cell movements, but the details are still unclear.

TFP proteins at the core of the main gene cluster include the major pilin, PilA1, the pre-pilin peptidase PilD, assembly and disassembly ATPases, PilB and PilT, as well as other pilins decorating the main pili structure, known as minor pilins. The three minor pilins on main gene cluster, PilK, PilU and PilV, have been proposed to be required for pili formation. However, their locations on the pili and the precise function are still unknown. Other unrelated minor pilins have been reported: PilJ, which was the first pilin to have its structure determined and has been shown to incorporate into the pili filament, and pilW. However, their biological importance remains unclear.

Here, we report our work on the role of different TFP proteins in *Clostridium difficile*. The effect on bacterial growth motility, cell-surface attachment and biofilm formation abilities of deleting the minor pilins genes in *Clostridium difficile* was investigated. The initial focus has been on the minor pilins in the main TFP locus - pilK, pilU, pilV - but the more distant minor pilins, pilJ and pilW, were also studied. Together, our results contribute to our growing understanding how the different pilin subunits affect pili formation and provide new insights on the role of TFP in *Clostridium difficile*.

P28

GENOME ORGANIZATION DRIVES THE TRANSCRIPTIONAL RESPONSES TO SUB-INHIBITORY CONCENTRATIONS OF POLYMERASE INHIBITORS IN *Clostridium difficile*

I.M. Boekhoud[1], E. van Eijk[1], E.J. Kuijper[1], I.M.J.G. Sanders[1], G.E. Wright[2], W.K.Smits[1]

1 Leiden University Medical Center (LUMC), Department of Medical Microbiology, PO Box 9600, 2300 RC, Leiden, The Netherlands 2 GL Synthesis Inc., One Innovation Drive, Worcester, MA 01605, USA

Background: Antimicrobial exposure can alter transcription patterns, potentially leading to tolerance and/or increased resistance. DNA polymerase inhibitors can modify gene expression resulting from an altered origin (oriC):terminus (terC) ratio [1]. Through the increase in oriC:terC ratio, an origin-proximal transcription factor, such as the general stress response regulator sigma factor B (σB), can be upregulated causing indirect secondary effects. In this research, we describe the adaptational response of *Clostridium difficile* to DNA polymerase (PolC) inhibition.

Methods: RNA-Seq analysis was performed on strain 630Δerm exposed to sub-inhibitory concentrations of the PolC inhibitors HPUra and 362E (N2-(3,4-Dichlorobenzyl)-7-(2-[1-morpholinyl]ethyl)guanine; MorE-DCBG) [2]. Marker Frequency Analysis was performed to measure changes in oriC:terC ratio. To validate the RNA-seq analysis and to study the effect of σ^B on the transcriptional response resulting from PolC inhibition, luciferase reporter constructs were analyzed in a wild type and a sigB knockout strain.

Conclusions: The strongest transcriptional response was observed for cells exposed to 362E. Compared to HPUra, 362E showed a larger increase in oriC:terC ratio resulting from relative up-regulation of origin-proximal genes (10- vs 3-fold increase). This increase in oriC:terC ratio was not observed for other antimicrobials with a different mechanism of action, such as metronidazole, fidaxomicin and surotomycin. The luciferase assay demonstrated that part of the increase in transcriptional activity resulting from 362E treatment is mediated by σ^B. These results suggest that locating stress response genes near the origin could be a conserved adaptive strategy for bacteria to cope with DNA replication inhibition.

1. Slager, J., et al., Antibiotic-induced replication stress triggers bacterial competence by increasing gene dosage near the origin. Cell, 2014. 157(2): p. 395-406.
2. Dvoskin, S., et al., A novel agent effective against *Clostridium difficile* infection. Antimicrob Agents Chemother, 2012. 56(3): p. 1624-6.

P29

PROTEOLYTIC ACTIVITY OF *Clostridium difficile* HTRA IS IMPORTANT FOR HTRA EXPRESSION LEVELS, BUT NOT FOR SPORULATION

Jeroen Corver[1], Tatiana M. Shamorkina[1,2] and Paul J. Hensbergen[2]

[1]Experimental Bacteriology, Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands; [2]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands.

Background and aims : The *Clostridium difficile* genome encodes one homolog of the HtrA serine protease/foldase. In many bacteria, HtrA is important for virulence, mainly because it is involved in the stress response and thus survival of the bacteria in the host. Consequently, strains that lack a functional HtrA are highly attenuated.

In contrast, we have previously shown that the *Clostridium difficile* HtrA knockout is more virulent in hamsters, likely because of an increase of toxin levels. The knockout also displayed lower sporulation and diminished binding to target cells.

Methods and Aims: To dissect the relative importance of HtrA's protease vs. foldase activity for one or more of these phenotypes, we have made a *Clostridium difficile* mutant strain in which only the proteolytic activity of HtrA was abolished (S217A) and have measured sporulation activity and analyzed total protein content to find differences between HtrA WT, knockout and mutants strains.

Results : Interestingly, we found that in the S217A mutant strain, HtrA was highly over expressed compared to the wild type strain, as evidenced by W-blot and a luciferase reporter assay. So, a lack of HtrA proteolytic activity led to a large increase in htrA transcription. In addition, we found that sporulation of the S217A mutant was unaffected, showing an important role for the foldase activity of HtrA in sporulation.

Comparative proteomic analysis of WT, S217A mutant and HtrA knockout strains showed that other proteins were also highly up regulated in the mutant and the knockout strain, of which the uncharacterized proteins CD2242 and CD2243 (two Zinc-finger domain-containing proteins) were the most prominent. To understand the link between the up regulation of these proteins and HtrA , we are currently investigating what proteins bind to the promoters of htrA and cd2243.

Furthermore, through proteomic analysis of conditioned medium of growing cells, we are analyzing alterations in the levels of secreted proteins from wild type, S217A mutant and HtrA knockout cells.

Conclusions: HtrA proteolytic activity is not needed for sporulation of C.difficile, but lack of proteolytic activity leads to a dramatic increase of htrA transcription. Several other proteins are highly upregulated in absence of HtrA proteolytic activity, among which CD2242 and CD2243 are the most prominent.

P30

IDENTIFICATION AND VALIDATION OF TWO PEPTIDE MARKERS FOR THE RECOGNITION OF *Clostridium difficile* MLST-1 AND MLST-11 BY MALDI-MS

Jeroen Corver[1,2], Jeff Sen[3], Bastian V.H. Hornung[1,2], Bart J. Mertens[4], Eric K.L. Berssenbrugge[1], Ingrid M.J.G. Sanders[1], Nitin Kumar[5], Trevor Lawley[5], Ed J. Kuijper[1,2], Paul J. Hensbergen[3], Simone Nicolardi[3]

[1] Leiden University Medical Center, Center of Infectious Diseases, Department Medical Microbiology, section Experimental Bacteriology 2333 ZA Leiden, Netherlands; [2] Center for Microbiota Analysis and Therapy, Department Medical Microbiology, 2333 ZA Leiden University;[3] Leiden University Medical Center, Center for Proteomics and Metabolomics, 2333 ZA Leiden, Netherlands; [4] Leiden University Medical Center, Department of Medical Statistics and Bioinformatics, 2333 ZA Leiden, Netherlands; [5] Host-Microbiota Interactions Laboratory, Wellcome Sanger Institute, Hinxton, CB10 1SA, UK.

In clinical microbiology laboratories, *Clostridium difficile* is commonly identified by MALDI-TOF MS while subsequent DNA-based typing methods, such as Multi Locus Sequence Typing and PCR-ribotyping, are used to discriminate *Clostridium difficile* strains. The first method is fast, cheap and easy to perform while the last two techniques are more cumbersome and time-consuming. Hence, there is a large interest in developing MALDI-TOF MS methods that could directly provide information on *Clostridium difficile* strains. However, the relatively low resolution of MALDI-TOF MS spectra has hitherto hampered the straightforward applicability of this technique for typing purposes. For this reason, in this study, we used ultrahigh resolution Fourier transform ion cyclotron resonance (FTICR) MS to generate more detailed and comprehensive bacterial protein profiles. This resulted in the identification of potential peptide markers for the recognition of *Clostridium difficile* MLST-1 (RT-027) and MLST-11 (RT-078). The elucidation of the amino acid sequence of these markers allowed the mining of a large publically available genome database (i.e. 2689 genomes) of *Clostridium difficile* MLSTs, for the accurate determination of their sensitivity and specificity. In addition, following this bioinformatics approach, a peptide marker specific for MLST-15 (RT-010) was also found.

The use of MALDI-FTICR MS for routine applications in the clinical microbiology laboratories is nowadays not feasible because of its high costs. For this reason, we developed a fast and simple enrichment/purification method which allows the unambiguous identification of the MLST-1 (RT-027) marker also on a lower resolution MALDI-TOF MS platform making this method suitable for clinical applications for example in case of a hospital outbreak of *Clostridium difficile* MLST-1 strains, when prompt management of bacterial contamination and infection control is required.

S-LAYER SECRETION IN *Clostridium difficile*

Peter Oatley, Robert Fagan

Department of Molecular Biology and Biotechnology, University of Sheffield, United Kingdom

Clostridium difficile is surrounded by a protective surface-layer (S-layer) that is essential for its virulence. The S-layer is a paracrystaline, proteinaceous complex that is constructed over the peptidoglycan cell wall, where it is involved in extracellular interactions and acts as a semi permeable barrier. S-layer consists of two proteins; S-layer protein (SlpA) and cell wall protein V (CwpV). Being at the surface of the cell and having a crucial role in *Clostridium difficile* infection (CDI), S-layer is a common target for anti *Clostridium difficile* therapeutics, yet little is known about how its components are secreted or assembled at the surface. The aim of my research has been to understand the route of SlpA export and quality control and to uncover novel targets for CDI intervention. Crosslinking experiments using partially translocated SlpA and the core secretory ATPases of *Clostridium difficile*, SecA1 and SecA2, together with proteomics have revealed additional factors involved in S-layer biogenesis. In addition to this, fluorescence microscopy has been used to highlight sites of SlpA secretion and label areas of newly formed S-layer during cell growth and division to better understand the steps involved in the coordinated secretion of a stable, functional S-layer.

P32

Clostridium difficile CELL SURFACE BIOGENESIS

Shauna O'Beirne, Robert P. Fagan.

University of Sheffield, Department of Molecular Biology and Biotechnology, United Kingdom.

On the *Clostridium difficile* cell surface is a proteinaceous paracrystalline array, known as the S-layer. The S-layer of *Clostridium difficile* is composed of two proteins: the high molecular weight (HMW) and the low molecular weight (LMW) protein, derived from the pre-protein SlpA. PS-II, an anionic polymer found in all *Clostridium difficile* strains examined to date, has been identified as the ligand responsible for the attachment of S-layer and associated cell wall proteins.

Previous efforts to knock out slpA have proved unsuccessful. The genes thought to encode the PS-II synthesis pathway are also thought to be essential. However, by using bacteriocins that specifically target the S-layer, we recently isolated a mutant which had no evident S-layer due to a mutation in the slpA gene. As the S-layer was previously thought to be essential, it now brings into question whether PS-II is also essential. In the strain lacking an S-layer, we have now created a deletion mutant in the putative PS-II polymerase and we are attempting to generate additional mutations in the polysaccharide synthesis pathway. Analysis of these mutants will provide insights into the mechanism of PS-II synthesis and shed light on its function in cell morphogenesis.

P33

IDENTIFICATION OF GENES IMPLICATED IN COLONISATION AND SURVIVAL OF *Clostridium difficile* IN THE GUT, USING RANDOM TRANSPOSON MUTAGENESIS

Nadia J. Fernandes, Roy R. Chaudhuri, Robert P. Fagan

University of Sheffield, Faculty of Science, Molecular Biology and Biotechnology Department, Sheffield, United Kingdom

Little is known about the mechanisms by which *Clostridium difficile* colonises and persists within the gut environment, or the signals required for the expression of its toxin genes. As we know, the available methods of producing single deletion *Clostridium difficile* mutants are rather time consuming. We set out to determine genes essential for survival under a variety of in vivo and in vitro conditions, using high-throughput mutagenesis.

Transposon directed insertion site sequencing (TraDIS) was combined with high-density transposon mutagenesis to generate over 70,000 unique *Clostridium difficile* mutants. By analysing the fitness of these mutants during their growth cycle, we elucidated genes essential for growth and sporulation in vitro. We have further exploited this method to assess gene fitness of a pool of *Clostridium difficile* mutants in the mouse gut, thereby allowing us to gain insights into the basic biology of the bacterium. A total of 62 in vivo datasets and 16 in vitro data sets have been analysed, one of the largest studies of its kind, and production if new libraries is underway.

Hopefully, this high-throughput approach will help decipher the functionality of the *Clostridium difficile* genome quicker and with greater ease. Larger data sets would enable us to fine tune this method and improve accuracy and rate of data analysis.

P34

THE HISTONE-LIKE PROTEIN HUPA OF *Clostridium difficile* COMPACTS DNA

Ana Paiva[1], Annemieke Friggen[1], Roxanne Douwes[1], Liang Qin[2], Remus Dame[2], Wiep Klaas Smits[1]*

[1]Leiden University Medical Center, Leiden, Netherlands; [2]Faculty of Science, Leiden Institute of Chemistry, Leiden, Netherlands

Background and aim. The maintenance and (re-)organization of the chromosome plays an important role in the development and survival of bacteria. Histone-like proteins are architectural proteins that bind DNA, modulate its conformation and by doing so affect a variety of cellular processes. No histone-like proteins of *Clostridium difficile* have been characterized to date. Here, we investigate aspects of the *Clostridium difficile* HupA protein, a homologue of the histone-like HUα and β proteins of Escherichia coli.

Methods. We investigate the multimeric state of HupA using size exclusion chromatography and in vivo protein-protein interaction assays. DNA binding was investigated using electrophoretic mobility shift assays and tethered particle motion experiments. Localization was investigated using fluorescence microscopy.

Results and conclusion. HupA is a 10 kDa protein that is present as a homodimer in vitro and in vivo. HupA co-localize with the nucleoid of *Clostridium difficile*. It binds to the DNA without strong sequence specificity. Upon DNA binding, HupA induces a conformational change of the substrate DNA and leads to compaction of the chromosome.

The present study is the first to characterize a histone-like protein in *Clostridium difficile* and opens the way to study the role of chromosomal organization in DNA metabolism and other cellular processes.

INTERACTOMIC STUDIES OF PROTEINS INVOLVED IN THE STICKLAND FERMENTATION OF *Clostridioides difficile*

P35

K. E. Rennhack[1], D. Jahn[2] and J. M. Borrero de Acuña[1]

[1]TU Braunschweig, Institute of Microbiology, Braunschweig, Germany; [2]TU Braunschweig, BRICS Braunschweig Integrated Centre of Systems Biology, Institute of Microbiology, Braunschweig, Germany

The anaerobic, Gram-positive bacterium Clostridioides difficile is causing a severe diarrhoea which leads to several thousand deaths per year in Germany. Stickland fermentation of amino acids represents the most prominent energetic generation process for *Clostridium difficile* growth. It has been unequivocally correlated to toxin production. This metabolic pathway employs pairs of amino acids as electron acceptor-donor systems for generating ATP. The proton motive force and its consequent ATP synthesis appears on the proton-translocating ferredoxin:NAD+ oxidoreductase complex (Rnf), whose proton pump functioning is coupled to the reduction performance of reductases. Hence, our group strives toward the elucidation of protein-protein-interactions involved in the Stickland pathway. For that, interactomic techniques encompassing affinity co-purification of bait-prey protein complexes and proteomics-based strategies for the identification of interaction partners where chosen as scientific approach. At first the membrane associated RnfC subunit, which is part of the Rnf pump and the cytoplasmic PrdA monomer of the D-proline reductase complex will be subjected to interactomic studies. Their strep-II tagged versions will be employed as baits to capture the potential preys stabilizing the transient protein-interactions by in vivo cross-linking. Preliminary experiments have been conducted leading to the construction of *Clostridium difficile* 630Δerm strains harbouring pMTL82151rnfC-strep and pMTL82151prdA-strep whose genes are flanked by their naturally predicted promoter regions. The production of both, RnfC-strep and PrdA-strep, via affinity purification was confirmed by western blot analysis through Strep-tagII detection. Furthermore, the directed mutagenesis technology of ClosTron Group II intron was harnessed in order to inactivate the rnfC gene. This resulted in the generation of the 630Δerm rnfC::ermr mutant strain, which was further employed for complementation with the plasmid pMTL82151rnfC-strep. In conclusion the construction of background mutant strains for interactomic studies and preliminary experiments on the purification of the fusion protein RnfC-Strep-tagII have been successfully conducted. Subsequently, in vivo cross-linking experiments aiming at capturing protein interaction partners will be performed.

P36

MOLECULAR CHARACTERIZATION AND COMPARATIVE STUDY OF SPORULATION OF A NEW STRAIN OF *Clostridium difficile* MLST CLADE 2 ISOLATED AT A CANCER HOSPITAL IN BRAZIL

Cecília Leite Costa[1,2], Carlos Quesada-Goméz[3], César Rodríguez[3], Cibele B. M. Carvalho[2], Gerly Anne de C. Brito[1]

[1] Núcleo de Microscopia e Processamento de Imagens (NEMPI), Department of Morphology, Federal University of Ceará, Fortaleza, Brazil
[2] Laboratory of Bacteriology, Department of Pathology, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil
[3] Facultad de Microbiología and Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, San José, Costa Rica

The epidemiology of *Clostridium difficile* infections is dynamic and this is due to strains that continue to appear throughout the world. This study presents a detailed analysis of a new strain of *Clostridium difficile* (ICC45) isolated from a cancer patient hospitalized at Haroldo Juaçaba Hospital, Fortaleza-CE, Brazil. The strain was isolated from a 34-years old female patient diagnosed with breast cancer and metastasis to the nervous system with fatal outcome after negative detection of toxins A/B from feces by ELISA, followed by culture in CCFA, phenotypic identification, detection of toxin and tpi fragment genes by PCR and susceptibility to 8 antimicrobials by E-test. Molecular identification analyzes were performed by means of PFGE, PCR ribotyping, toxinotyping, whole genome and Multilocus sequence typing (MLST). Analyzes for the evaluation of sporulation of ICC45 were performed by Optical Microscopy through Gram staining, Scanning Electron Microscopy (SEM) and relative gene expression of spo0A by qPCR using SYBR Green. Relative gene expression was determined using the 2-ΔΔCt method. The 16S gene was used as internal control. ICC45 strain was assigned a new PCR ribotype and new pulsotype and was classified as ST41 of MLST Clade 2 and toxinotype IXb. ICC45 encodes a TcdB variant. Unlike NAP1/027, which also belongs to MLST Clade 2, ICC45 is sensitive to fluoroquinolones and has no overproduction of toxins A and B within 24 hours of culture, but express gene for binary toxin. The results of the qualitative evaluation suggest that the ICC45 strain forms more spores than the NAP1 and ATCC strains. In culture, the ICC45 strain forms a clearer branching around its colony on the blood agar plate which was not observed in the other strains, in culture of 7 days. This branching when viewed under optical microscopy and SEM shows innumerable loose spores and bacilli in sporulation. In the analysis of gene expression in 24h culture, the ICC45 strain showed higher expression ($p<0.0002$) of spo0A compared to NAP1 (R20291 and LIBA5756) and ATCC700057. No significant differences were found in the expression of spo0A between NAP1 and ATCC. The data presented here highlight the importance of studying the epidemiological situation and the understanding of sporulation by new strains of *Clostridium difficile* in order to improve the therapeutic and preventive strategies of CDI in hospital settings.

P37

STRUCTURAL INSIGHT INTO THE CELL WALL-ANCHORING MODULE OF *Clostridium difficile* S-LAYER PROTEINS

Aleksandra Usenik[1], Miha Renko[1,2,3], Marko Mihelič[1,2], Nataša Lindič[1], Jure Borišek[4], Andrej Perdih[4], Gregor Pretnar[1,2], Uwe Müller[5,6] and Dušan Turk[1,2]

[1] Department of biochemistry, molecular and structural biology, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia
[2] Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Jamova cesta 39, 1000 Ljubljana, Slovenia
[3] present address: MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge, CB2 0QH, UK
[4] National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia
[5] Macromolecular Crystallography Group, Helmholtz Zentrum for Materials and Energy Berlin, Albert-Einstein-Str. 15, D-12489 Berlin, Germany
[6] present address: Lund University, Synchrotron Radiation Research, Department of Physics, Sölvegatan 14, SE-223 62 Lund, Sweden

Background and aims: In Gram-positive bacteria, there are several types of cell wall proteins non-covalently attached via cell wall binding domains, in some cases forming surface protein layers (S-layers). Of the two evolutionary conserved S-layer-anchoring modules composed of three tandem SLH or CWB2 domains (PF04122), the latter is present in *Clostridium difficile*, one of the most important nosocomial pathogens. *Clostridium difficile* 630 contains 29 cell wall proteins (CWPs) that share the CWB2 module that mediates non-covalent binding to the secondary polymer PSII. We expect that structural insight will enable us to understand the functions of these proteins when acting alone and when assembled in the S-layer. The first two crystal structures we determined reveal the CWB2 module and its conserved surface residues that are likely involved in the attachment to the cell wall components. Methods: The mature Cwp8 and Cwp6 from *Clostridium difficile* 630 were overexpressed in Escherichia coli BL21(DE3), purified by Ni-affinity and size exclusion chromatography and crystallized by sitting-drop vapor-diffusion technique using optimised commercial screens. Platinum derivatives of the Cwp8 crystals were prepared by soaking. The crystal structures of Cwp8 and Cwp6 were determined by single-wavelength anomalous dispersion method and molecular replacement, respectively. Results: The crystal structures of Cwp8 and Cwp6 reveal multi-domain proteins, each with embedded triangular, disk-shaped trimer of Rossmann-fold CWB2 domains where the first domain of the trimer is composed of two fragments, one from the N- and the other from the C-terminal region. The N-terminal parts of Cwp8 and SlpA are structurally similar. Cwp6 contains an amidase domain with the fold of Amidase_3 family (PF01520) that degrades peptidoglycan from Staphylococcus aureus. Conclusions: The highly conserved residues shared between the CWB2 and SLH S-layer modules suggests a common or convergent evolutionary origin. The structural and sequence analysis of the CWB2 modules predicts that the PSII-binding site resides in the conserved grooves at the upper side of the CWB2 disc. Comparison of the Cwp8 structure with the dimensions of the S-layer lattices revealed by previously reported EM and AFM data suggests that *Clostridium difficile* S-layers are complex oligomeric structures, likely composed of several different proteins.

P38

FACTORS AFFECTING PRODUCTION OF THE BACTERIOSTATIC COMPOUND PARA-CRESOL IN *Clostridium difficile*

Mark A Harrison[1], Brendan W Wren[1] and Lisa F Dawson[1]

[1]Pathogen Molecular Biology Department, London School of Hygiene and Tropical Medicine

Clostridium difficile is a gram positive, spore forming anaerobe and the major cause of antibiotic associated diarrhoea worldwide. Approximately 20-30% of those infected with *Clostridium difficile* will suffer from relapse with the rate of relapse increasing upon each recurrence. It is well understood that *Clostridium difficile* infection occurs in patients where the gut microbiome has been disturbed resulting in a loss of colonisation resistance to *Clostridium difficile*. It has been shown that *Clostridium difficile*'s ability to produce the phenolic, bacteriostatic compound para-cresol potentially provides it with an advantage over species of the gut microflora and may contribute to infection recurrence by maintenance of dysbiosis. Production of p-cresol occurs from the fermentation of tyrosine via the intermediate para-hydroxyphenylacetate (p-HPA). p-HPA is converted to p-cresol via the actions of p-HPA decarboxylase encoded by the hpdBCA operon. Currently, very little is known about the factors that influence p-cresol production, other than we have shown that p-cresol production is low in rich media (Brain Heart Infusion) and significantly higher in less rich media (Yeast Peptone). Here we outline methods used to investigate factors involved in p-cresol production. We have utilised two complementary reporter systems to ascertain expression of the p-HPA decarboxylase by creating a promoter fusion using the upstream region of hpdBCA. The first reporter, a SNAP Tag, is a mutant of DNA repair protein O6-alkylguanine-DNA alkyltransferase, which can be bound and visualized using a fluorescent substrate. The second is a glucuronidase gene (GusA) from Escherichia coli, which hydrolyses substrates to give a product measurable by spectrophotometry. Through use of the SNAP Tag reporter we demonstrate that addition of p-HPA to *Clostridium difficile* cultured in minimal media results in significantly increased expression of the hpdBCA operon. We also found that addition of tyrosine has minimal effect on expression from the hpdBCA promoter. This suggests that a key driving factor behind expression of the hpdBCA operon is the level of p-HPA present. We plan to confirm these findings using the GusA reporter and investigate the mechanism by which p-HPA increases expression of the hpdBCA operon and how this may contribute to *Clostridium difficile* virulence through production of p-cresol.

P39

GENERATION OF A SNP-FREE, FULLY ERYTHROMYCIN-SENSITIVE *Clostridium difficile* 630 STRAIN USING CRISPR-CAS9 MUTAGENESIS

Patrick Ingle[1], Daphne Groothuis[1], Peter Rowe[2], Chris Humphries[1], Sarah Kuehne[3], Nigel Minton[1].

[1] BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), Centre for Biomolecular Sciences, Science Road, University Park, University of Nottingham, NG7 2RD, UK
[2] In Ovo B. V., J.H. Oortweg 19, 2333 CH Leiden, NL.
[3]School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B5 7SA, UK

Clostridioides difficile strain 630 was isolated from a patient with severe pseudomembranous colitis in Zurich, Switzerland in 1982. This strain became the model organism for studies of *Clostridium difficile* and was the first *Clostridium difficile* strain to have its genome sequenced in 2006. The generation of two, independent erythromycin-sensitive derivatives of strain 630 (630Δerm and 630E) facilitated reverse genetics studies via ClosTron insertional mutagenesis. These strains, generated via repeated subculture, both contain a 2.4 kb deletion in the mobilizable transposon Tn5398, removing one of two sequence-identical erm(B) genes present in 630. The loss of only erm2(B) was sufficient to confer erythromycin sensitivity, however reversion to erythromycin resistance was seen at low frequencies. A recent study identified multiple single nucleotide polymorphisms (SNPs) in the 630Δerm and 630E genomes and suggested these SNPs were responsible for conflicting reports on the relative importance of each toxin in *Clostridium difficile*-associated disease. Hence, we sought to remake an erythromycin-sensitive strain of 630 by removing both copies of the erm(B) using CRISPR-Cas9 genome editing to avoid SNP accumulation.

We developed three CRIPSR-Cas9 genome editing vectors, each containing a guide RNA targeting unique sequences within the Tn5398 target site. These vectors were conjugated into a newly acquired *Clostridium difficile* 630 reference strain from the NCTC culture collection (NCTC 13307) and colonies screened for the desired deletion using colony PCRs. Editing efficiencies of 96% were observed and after confirming plasmid loss the mutant strain was designated 630Δerm*. Whole genome sequencing of the parental NCTC 13307 and three independently generated 630Δerm* mutants revealed no additional SNPs were accumulated in one mutant strain. We further exemplified our CRISPR-Cas9 genome editing system by generating 630Δerm*ΔpyrE, again observing highly efficient genome editing and the accumulation of no additional SNPs during CRISPR-Cas9 mutagenesis.

CRISPR-Cas9 mutagenesis offers fast, precise genome editing requiring fewer restreaks than previous clostridial mutagenesis methods, therefore minimizing the risk of SNPs accumulating. Our fully erythromycin-sensitive 630Δerm* strain differs only from the NCTC 13307 reference strain via a 3.6 kb deletion of erm1(B) and erm2(B) from Tn5398.

P40

A *Clostridium difficile* BIOFILM: REMODELLING METABOLISM AND CELL SURFACE TO BUILD A 3D ARCHITECTURE

Isabelle Poquet [1,2,], Laure Saujet [2,3], Alexis Canette [1,^], Marc Monot[2,3], Jovanna Mihajlovic [4], Jean-Marc Ghigo [4], Olga Soutourina [2,3,#], Romain Briandet [1], Isabelle Martin-Verstraete [2,3] and Bruno Dupuy [2,3]*

*[1]Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France ; [2]Laboratoire de Pathogenèse des Bacteries Anaerobies, Institut Pasteur, 75724 Paris Cedex, France ; [3]Université Paris Diderot, Sorbonne Paris Cité, 75013 Paris, France ; [4]Unité de Génétique des Biofilms, Institut Pasteur, 75724 Paris Cedex, France ; ^Present address: IBPS Institute, Sorbonne Université, CNRS, 75005 Paris, France ; #Present address: Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, 91198, Gif-sur-Yvette cedex, France ; *Corresponding author*

Clostridium difficile is an entero-pathogen causing post-antibiotic diarrhea upon microbiota dysbiosis. Although biofilms could contribute to colonization, little is known about their development and physiology.

Different systems for biofilm growth in TYt medium were set up: continuous-flow micro-fermentors and 24- and 96-well micro-titration plates. Gene expression was studied by transcriptomics and qRT-PCR, and metabolic end-products by Gas Phase Chromatography. Cell morphology and biofilm architecture were observed by Light Transmission and Confocal Laser Scanning Microscopy.

Strain 630Δerm is able to form, in micro-fermentors, macro-colonies and submersed biofilms loosely adhesive to the glass surface. According to gene expression data, in biofilm cells, central metabolism is active and fuels fatty acid biosynthesis rather than fermentations. In agreement with expression data, succinate is consumed and butyrate production is reduced in biofilm cells. Toxin A expression, which is coordinate to metabolism, is consistently down-regulated, while several surface proteins and primary Type IV pili are over-expressed. C-di-GMP level is probably tightly controlled through the expression of several diguanylate cyclases and phosphodiesterases and might be high in biofilms cells. A Bacillus subtilis SinR-like regulator, CD2214, and/or CD2215, another regulator co-encoded in the same operon as CD2214, control many genes differentially expressed in biofilm. A submersed biofilm loosely adhesive to polystyrene is also formed in micro-titer plates. After recovery and fixation, biofilm cells are found to be embedded into a polymorphic material forming huge aggregates. The intact biofilm, when observed in situ, displays a 3D sparse and high architecture containing micro-aggregates. This architecture is affected by the inactivation of CD2214 and CD2215 genes, but not of two genes that are positively controlled by both CD2214-CD2215 and c-di-GMP and that encode the surface adhesin CD2831 and the major Type IV pilin PilA1. This thorough analysis of gene expression reprogramming and architecture remodelling in biofilm lays the foundation for a deeper understanding of this lifestyle and could lead to novel strategies to limit *Clostridium difficile* spread.

P41

INVOLVEMENT OF PERR AND ITS OPERON IN THE OXIDATIVE STRESS RESPONSE IN *Clostridioides difficile*

Daniel Troitzsch[1], *Silvia Dittmann*[1], *Susanne Sievers*[1]

[1]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*

Background and aims
Clostridium difficile is a vast problem in human health care, as it causes serious and recurrent inflammation of the intestinal epithelium often with a lethal outcome. It is considered to be a strictly anaerobic bacterium, therefore the presence of oxygen and reactive oxygen species should hamper its growth. However, previous studies have shown a high, strain-dependent oxygen tolerance of the pathogen. To understand the high tolerance of *Clostridium difficile* to oxidative conditions we will identify and characterize candidates in the genome of *Clostridium difficile*, which are involved in the oxidative stress response.

Methods
A genome-wide analysis was performed to find potential oxidative stress regulators. To substantiate results the expression of selected genes is tested in Northern blot analysis and deletion mutants are phenotypically characterized. A special focus is set on perR and its operon, but also on the entity of the oxidative stress induced PerR regulon.

Results
Screening of the *Clostridium difficile* genome brought to light several candidates putatively involved in the oxidative stress response and described in other bacteria to mediate tolerance against oxidative stress. In particular a ruberythrin, encoded by rbr1, is highly abundant in *Clostridium difficile* 630 Δerm. The gene is located in an operon together with the genes for the transcriptional repressor PerR; a desulfoferrodoxin, a class III superoxide reductase and a glutamate dehydrogenase, which features a rubredoxin like structure at the N-terminus. The operon is apparently highly expressed since all gene products are very abundant in the late exponential phase of growth.

Conclusion
Clostridium difficile has a broad variety of so far poorly characterized players potentially involved in the oxidative stress response. This study sheds light on the function and interplay of proteins involved in detoxification of oxygen and reactive oxygen species and is therefore a starting point for the abrogation of such adaptation mechanisms and with that for the development of novel treatment strategies.

P42

ADAPTATION OF *Clostridioides difficile* TO OSMOTIC STRESS

Annika-M. Michel[1], Tamara Hoffmann[2], Erhard Bremer[2], José M. Borrero-de Acuña[3] and Dieter Jahn[1]

[1]Technische Universität Braunschweig, Institute of Microbiology and Braunschweig Integrated Center of Systems Biology (BRICS), Braunschweig, Germany;[2]Philipps-Universität Marburg, Department of Biology, Laboratory for Microbiology, Marburg, Germany; [3]Technische Universität Braunschweig, Institute of Microbiology, Braunschweig, Germany

Little is known about the gene regulatory, protein interaction and metabolic networks underlying the host associated life cycle of *Clostridium difficile*. Here we are investigating the response of the bacterium to osmotic stress. Several osmoprotectant uptake (opu) transporters including proline transporter (put) play an important role in the protection of Bacillus subtilis against changing osmotic conditions [1]. Blast analyses identified gene homologues of B. subtilis opuC and putP in *Clostridium difficile*. To determine their functional role mutants deficient in the production of functional clostridial OpuC (CD630_09010) and PutP (CD630_35750) were generated and grown in minimal medium with increasing salt and sugar concentrations. Different osmolytes (e.g. glycine betaine, carnitine, choline) were tested for their contribution to osmoprotection and affinity to the transporter OpuC. To obtain a holistic view on the osmotic protection process a systems biology approach encompassing RNAseq, proteomics and metabolomics experiments will be performed. In order to characterize the immunological relevance macrophage assays as well as mouse infection assays will be carry out.

[1] Zaprasis A. et al. Osmoprotection of Bacillus subtilis through Import and Proteolysis of Proline-Containing Peptides. Appl Environ Microbiol. 2013 Jan; 79(2)

P43

OXYGEN TOLERANCE AND REACTIVE OXYGEN SPECIES PROTECTION IN *Clostridium difficile*

Alves Feliciano Carolina[1,2], Kint Nicolas [1,2], Folgosa Filipe[3], Martins Maria C.[3], Teixeira Miguel[3] , Dupuy Bruno[1,2], Martin-Verstraete Isabelle[1,2]

[1] Laboratoire Pathogenèse des Bactéries Anaérobies, Institut Pasteur, Paris, France ; [2] Université Paris Diderot, Sorbonne Paris Cité, Paris, France ;[3] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal.

After germination of *Clostridium difficile* spores in the gut, vegetative cells are exposed to several stresses such as antibiotics, bile salts, pH variations as well as reactive oxygen/nitrogen species (ROS/RNS) produced by the host immune system during inflammation. Furthermore, although the intestinal tract is regarded as mainly anoxic, low oxygen (O2) tensions are present in the large intestine and tend to increase after antibiotic treatments. To survive to this hostile oxidative environment, *Clostridium difficile* had to develop mechanisms of protection, detoxification pathways and repair systems. We recently showed that the alternative sigma factor of the general stress response, σB, plays an important role in the protection of *Clostridium difficile* against the different stresses the bacterium is facing inside the host including O2 and ROS. Among the genes positively controlled by σB, we identified genes encoding proteins likely involved in O2 tolerance and ROS detoxification, such as the two reverse rubrerythrins, CD1524 and CD1474, and the flavodiiron protein NorV. To determine their involvement in *Clostridium difficile* stress response, the genes encoding those proteins were inactivated. We showed that a norV mutant and a double CD1474 CD1524 mutant are less tolerant to low physiological O2 tension as shown for the sigB mutant. To functionally characterize these proteins, CD1474 and CD1524 and norV from *Clostridium difficile* were overexpressed in Escherichia coli and the 3 proteins were purified. We then demonstrated that the two reverse rubrerythrins have NADH-linked hydrogen peroxide- and O2-reductase activity, being H2O2 the preferred substrate. Furthermore, NorV mainly acts as an O2-reductase. In the present study, we showed that the reverse rubrerythrins and NorV play a crucial role protecting *Clostridium difficile* against O2 and oxidative stress.

P44

ELUCIDATION OF THE MECHANISTIC DETAILS OF *Clostridioides difficile's* TOLERANCE TO HIGH CONCENTRATIONS OF DIFFERENT BILE ACIDS

Nicole Metzendorf, Silvia Dittmann, Christian Wolff, Daniela Zühlke, Rabea Schlüter, Katharina Riedel, Susanne Sievers

University of Greifswald, Center for Functional Genomics of Microbes (CFGM), Institute of Microbiology, Greifswald, Germany

Background and aims
Clostridium difficile is an opportunistic intestinal human pathogen that requires depleted microbiota to cause an infection and has become one of the most serious hospital-acquired pathogens. It is known, that the composition of the individual bile acids cocktail has a great impact on the susceptibility towards *Clostridium difficile* infections. Especially secondary bile acids produced by intestinal bacteria hamper *Clostridium difficile* proliferation.

We conducted proteomics analyses to elucidate the molecular mechanisms of the differential effects single bile acids have on vegetative cells of *Clostridium difficile*.

Methods
Growth-diminishing concentrations of the main bile acids (cholic-, chenodeoxycholic-, deoxycholic- and lithocholic acid) were determined in shock experiments and during long-term challenges, respectively. An LC-MS/MS-based proteomics approach was used to record stress signatures for all four bile acids. The data was analyzed and visualized via PCA, Voronoi treemaps, heatmaps and volcano plots. Motility assays were performed on swimming agar plates, and morphological alterations tracked and quantified by negative contrast electron microscopy.

Results
Inhibitory concentrations for the single bile acids vary significantly. A general overlapping stress response was observed for all tested bile acids, which may be due to the common steroid structure and overlapping chemical properties. However, several proteins showed an altered abundance in the presence of only a single or a few of the bile acids, indicating the existence of specific stress responses. For instance, proteomics revealed a reduction of structural proteins of flagella primarily in cells grown in the presence of chenodeoxycholic- and lithocholic acid, a finding that was supported by the observation that these bacteria also had fewer/shorter flagella (electron microscopy) and a reduced motility.

Conclusion
From the specific stress response patterns of different bile acids, one can assume ligand-receptor like interactions of single bile acids and bacterial proteins. Abrogating such interactions could impede *Clostridium difficile*'s adaptation to bile acids which could be a starting point for novel treatment strategies.

THE DIVERSE ROLES OF CYCLIC-DI-AMP IN *Clostridium difficile*

P45

Peltier Johann[1,2], *Flor Saporta*[1,2], *Kaever Volkhard*[3], *Dupuy Bruno*[1,2]

[1]Laboratoire Pathogenèse des Bactéries Anaérobies, Institut Pasteur, Paris, France; [2]Université Paris-Diderot, Paris, France; [3]Research Core Unit Metabolomics, Hannover Medical School, Hannover, Germany

Nucleotide signaling molecules are important messengers in key pathways of cellular responses to varied environments. These molecules initiate signal transduction by binding to receptors (proteins or regulatory RNAs called riboswitches) to regulate downstream cellular processes. Among the nucleotide signals, cyclic di-guanosine monophosphate (c-di-GMP) has been well characterized and is now recognized as a central regulator in bacterial cells controlling lifestyle selection. In contrast, the functions of the more recently discovered cyclic di-adenosine monophosphate (c-di-AMP) are less defined. C-di-AMP is predominantly produced in Gram-positive and is often essential for growth suggesting its involvement in key cellular functions.

In this work, we demonstrated for the first time that *Clostridium difficile* produces c-di-AMP in addition to c-di-GMP, unlike most bacteria that have specialized in using either one or the other messenger. We identified two diadenylate cyclases, DacA and DisA, producing c-di-AMP and a single phosphodiesterase GdpP degrading it. We individually deleted the corresponding genes and characterized the mutant strains. We found that c-di-AMP is implicated in bacterial growth, osmotolerance, cell wall antibiotic susceptibility and biofilm formation. Moreover, we found that increased levels of c-di-AMP impact expression of approximately 14% of the total genes, indicating that c-di-AMP regulates various cellular pathways. Surprisingly, our transcriptomic analysis also revealed that several riboswitches thought to specifically bind c-di-GMP might also respond to c-di-AMP, suggesting a cross-link between the two molecules.

By bioinformatics search, we identified 15 putative c-di-AMP binding proteins. We demonstrated that c-di-AMP binds to the KtrA and KdpD proteins, both involved in potassium (K^+) uptake. Moreover, we showed that a double dacA-disA mutant strain is viable only when bacteria are grown in media containing low K^+ concentrations. This result indicates that the control of K^+ homeostasis is an essential function of c-di-AMP in *Clostridium difficile*.

We also identified a transcriptional regulator as a c-di-AMP-binding protein. We are currently investigating the role of this regulator and further results will be discussed.

Collectively, our data highlight the important role of c-di-AMP in regulating fundamental cellular pathways in *Clostridium difficile*.

P46

DECIPHERING OF THE SIGMA B SIGNALLING ACTIVATION PATHWAY IN *Clostridium difficile*

Kint Nicolas[1,2], Alves Feliciano Carolina[1,2], Hamiot Audrey[1,2], Denic Milica[1,2], Dupuy Bruno[1,2] and Martin-Verstraete Isabelle[1,2].

[1] Laboratoire Pathogenèse des Bactéries Anaérobies, Institut Pasteur, Paris, France ; [2] Université Paris Diderot, Sorbonne Paris Cité, Paris, France

Inside the intestinal tract of the host, vegetative cells of C. difficile have to adapt to the stresses they cope with by inducing different protection, detoxification and repair systems. In *Clostridium difficile*, most of these systems belong to the general stress response involving the alternative sigma factor σB. However, less is known about the mechanisms involved in the activation of σB in C. difficile. In firmicutes, activation of σB relies on a post-translational regulatory mechanism called partner switching. Protein-protein interactions involved in this partner switching mechanism depend on the phosphorylated state of an anti-anti-sigma factor called RsbV. Specific PP2C phosphatases responding to environmental and energetic stresses are involved in the dephosphorylation process of RsbV that leads to the activation of σB. To determine if this mechanism is conserved in *Clostridium difficile*, we both inactivated rsbV and over-expressed the anti-sigma factor RsbW. In this work, we study the signalling pathway responsible for the activation of σB in *Clostridium difficile*. Contrary to other firmicutes, the expression of sigB is not auto-regulated in C. difficile and is constitutively expressed. We confirmed the conservation of the partner switching involved in σB activation and we identified the PP2C phosphatase CD2685, renamed RsbZ, involved in the dephosphorylation of RsbV. We identified that disruption of the proton motive force induced by CCCP treatment triggers σB activity in a RsbZ-dependent manner. Finally, we observed that σB is heterogeneously active in a subpopulation of cells as soon as the exponential phase in C. difficile likely leading to a "bet-hedging" strategy and allowing a better chance for the cells to survive to adverse conditions. In the present study, we showed that the σB signalling activation pathway in *Clostridium difficile* displays both conserved features with the one observed in firmicutes and original

P47

DEVELOPMENT AND TESTING OF A RECOMBINANT BCLA3 SPORE GLYCOPEPTIDE VACCINE FOR PREVENTION OF *Clostridium difficile* INFECTION

S. M. Logan, A. Aubry, E. Vinogradov, W. Zou, G. Harris, H. Zhou, W. Chen, M. Schur, M. Gilbert

Vaccine Program, Human Health Therapeutics Research Centre, National Research Council, Ottawa, Canada.

Spores of *Clostridium difficile* are the infectious agents responsible for the initial colonisation of the GI tract in CDI. As such, the surface components of spores present a key target for the development of therapies directed towards prevention of GI tract colonisation. We have shown previously that a component of *Clostridium difficile* spores, the BclA3 spore exosporangial protein, is glycosylated at multiple sites within the central collagen repeat region with a complex carbohydrate structure(1) and more recently that this glycan is immunogenic on the spore surface. We have expressed a *Clostridium difficile* sgtA glycosyltransferase gene which is involved in BclA3 glycan biosynthesis in E. coli as a soluble fusion protein. The functional characterisation and optimisation of in vitro reaction conditions for this novel glycosyltransferase enzyme was completed using a number of synthetic peptides from the collagen repeat region of the BclA3 protein. In vitro scale up synthesis of a BclA3 glycopeptide using the SgtA GT was performed, and we purified 10 mg of the glycopeptide product by HPLC. This glycopeptide (or corresponding peptide) was used in bromoacetyl conjugation chemistry to produce KLH-glycopeptide or KLH-peptide conjugates. The KLH-conjugates were then used to immunise rabbits and mice and the immune responses to each conjugate examined by ELISA using BclA3 peptides, BclA3 glycopeptides as well as *Clostridium difficile* spores. Robust immune responses were raised to both KLH-conjugates although only the KLH-glycopeptide immune serum was able to recognize spores demonstrating the significance of BclA3 glycosylation on the spore surface. The utility of the BclA3 glycopeptide conjugate as a vaccine candidate is currently under evaluation in both in vitro and in vivo models and results will be presented.

[1]Strong PC, Fulton KM, Aubry, A. Foote, S, Twine, SM and Logan S.M. 2014 J. Bacteriol, 196:2627-2637

P48

COMBATING *Clostridium difficile* INFECTION WITH AN OPTIMISED BACTERIOPHAGE COCKTAIL

Janet Yakubu Nale and Martha Rebecca Jane Clokie

Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK

Clostridium difficile infection (CDI) remains a major health challenge. This is due to the emergence and spread of hypervirulent antibiotic-resistant *Clostridium difficile* ribotypes, the bacterial spores and biofilms that are impermeable to antibiotics and the lack of sufficient treatment options. The drawbacks of current approved antibiotics for CDI relate to efficiency, cost and the potential to cause dysbiosis in the gut, which contributes to relapse and recurrent infection. Consequently, new antimicrobials are needed to combat CDI and we have developed a novel cocktail of well characterised broad host-range bacteriophages (viruses that specifically kill bacteria). This cocktail can efficiently target and kill clinically prevalent ribotypes in vitro and significantly reduce *Clostridium difficile* colonisation in vivo. I will present the efficacy of the cocktail to clear *Clostridium difficile* in the presence of competitive pressures from human gut microbiome. In this study, batch fermentation vessels spiked with combined faecal slurries from four healthy volunteers were inoculated with cultures of a clinical *Clostridium difficile* 014/020 isolate and treated with the phage cocktail. The impact of phage therapy on *Clostridium difficile* and other components of the gut microbiome were ascertained using viability assays and metagenomic sequencing. After a 5 h post-phage exposure approximately, 6-log reductions in *Clostridium difficile* abundance were observed following a prophylactic regimen and complete elimination was seen after 24 h in both phage prophylactic and remedial regimens. No detectable detrimental impact on gut commensal enterococci, bifidobacteria, lactobacilli, total anaerobes, and enterobacteria was observed in both regimens. However, a ~2-log increase in the enterobacteria, lactobacilli, and total anaerobe abundance was observed in the phage-only-treated control compared to other treatments. The viability data concurs with the metagenomics analysis. Clearly, the phage cocktail efficiently removed *Clostridium difficile* from the fermentation vessels and supported the proliferation of specific beneficial human gut commensals. Thus, the phages can contribute to restoring gut health and preventing *Clostridium difficile* colonisation and disease establishment. This study further provides convincing evidence to support the therapeutic development of phage cocktails to combat CDI in hospitals.

P49

FECAL MICROBIOTA TRANSPLANTATION FOR RECURRENT *Clostridium difficile* INFECTION: EXPERIENCE WITH LYOPHILIZED CAPSULES

Reigadas Elena[1,2,3], Olmedo María[1,3], Váquez-Cuesta Silvia[1,3], Valerio Maricela[1,2,3], Alcalá Luis, Marín Mercedes, Muñoz Patricia[1,2,3,4] and Bouza Emilio[2,3,4]

[1] Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain, [2] Medicine Department, School of Medicine, Universidad Complutense de Madrid (UCM), Madrid, Spain, [3] Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain, [4] CIBER de Enfermedades Respiratorias (CIBERES CB06/06/0058), Madrid, Spain

Background: Fecal microbiota transplantation (FMT) is a highly effective therapy for refractory and recurrent *Clostridium difficile* infection (CDI). Despite its excellent efficacy, FMT is still not a routine procedure in most European countries. We describe our experience with FMT to treat recurrent CDI, including the novel approach based on oral administered lyophilized fecal capsules.

Methods: A prospectively recorded single-center case series of patients with recurrent CDI treated with FMT between June 2014 and May 2018 was analyzed. Primary outcome was defined as CDI resolution without CDI recurrence in a two month period. FMT was administered via colonoscopy, nasoyeyunal tube, oral liquid capsules, oral lyophilized capsules. All stool donors were rigorously screened.

Results: FMT was performed in 22 patients with recurrent CDI. Median age was 71.9 and 63.6% were females. Eight FMT were performed via nasoyeyunal tube, 5 were performed via oral frozen líquid capsules,7 via lyophilized capsules and 2 were performed via colonoscopy. There were no procedure-related adverse events, except for bacteriemia in one patient, no complications were observed for lyophilized capsule administration. During the follow-up period, recurrence of CDI was observed in one patient at one month after FMT due to antibiotics. Primary cure rate was achieved in 81.0% of patients and the overall cure rate of FMT was 85.7%. FMT procedure by lyophilized capsules achieved 85.7% cure, oral liquid capsules 80%, colonoscopy 100% and 75.0% with nasoyeyunal tube.

Conclusions: In our cohort, FMT proved to be safe and effective even in high risk patients. Our initial clinical experience suggests that oral administration of FMT using lyophilized preparations also proved to be safe, well-tolerated and highly effective treatment for recurrent CDI. This administration method seems feasible in the routine of a hospital and will allow FMT to be more widely used.

P50

MECHANISMS OF ANTIBACTERIAL ACTION OF PLANT EXTRACTS AGAINST *Clostridium difficile*

Niloufar Roshan [1], Thomas V. Riley [1-4], Daniel R. Knight [3], Katherine A. Hammer [1]

[1]*School of Biomedical Sciences (M504), The University of Western Australia, 35 Stirling Hwy, Crawley, Western Australia, Australia, 6009.*
[2]*Division of Microbiology, PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Nedlands, Western Australia, Australia, 6009.*
[3]*School of Veterinary & Life Sciences, Murdoch University, Murdoch, Western Australia, Australia, 6150.*
[4]*School of Medical & Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia, 6027.*

Backgrounds and aims: *Clostridium difficile* causes disease ranging from self-limiting diarrhoea to severe pseudomembranous colitis. Antimicrobial treatment failures and patients with multiple recurrences have driven the search for new therapies. Some natural products have broad-spectrum antimicrobial activity with several showing activity against *Clostridium difficile*. Understanding the mechanism of action of these products is important to further characterise their efficacy. Thus, this study aimed to investigate the mechanism of action of five bactericidal products, cinnamon root powder, peppermint oil, trans-cinnamaldehyde, menthol and zingerone and four bacteriostatic products, fresh garlic bulb extract, garlic clove powder, allicin and Leptospermum honey against two *Clostridium difficile* strains.

Methods and results: As determined by measuring the optical density at 620 nm, none of the products caused bacteriolysis. The time-kill assay showed a > 3 log10 reduction in *Clostridium difficile* viable counts by all five bactericidal products after 24 h of exposure. An ATP-leakage assay showed that all five products at most concentrations significantly reduced the intracellular ATP after 1 h of incubation ($P \leq 0.01$). Alterations in cell permeability were assessed by measuring the leakage of 260-nm absorbing materials, protein leakage using Bradford assay and the propidium iodide uptake assay. All five bactericidal products damaged the cell membrane as seen in two or more cell permeability assays. The effect of three bacteriostatic products on protein synthesis was determined using an Escherichia coli S30 extract system, and only Leptospermum honey (16% w/v) showed inhibition of prokaryotic protein synthesis ($P < 0.01$). None of the products showed elevated minimum inhibitory concentrations against strains of *Clostridium difficile* harbouring DNA gyrase mutations, or conjugative transposons carrying ermB and tetM.

Conclusions: The findings indicate that damage to the cytoplasmic membrane may contribute to the mechanism of action of several natural products against *Clostridium difficile*. Also, the absence of cross-over mechanisms of resistance between standard antibiotics and natural products are suggested. Further studies are required to determine the efficacy of these products in vivo.

P51

TWO YEARS OF EXPERIENCES WITH TREATMENT OF *Clostridioides difficile* INFECTIONS USING THE NETHERLANDS DONOR FECES BANK

Karuna Vendrik[1], Elisabeth Terveer[1], Bastian Hornung[1], Rogier Ooijevaar[2], Abraham Goorhuis[3], Martijn Bauer[4], Els van Nood[5], Yvette van Beurden[2], Christina Vandenbroucke-Grauls[6], Hein Verspaget[7], Josbert Keller[8], Ed Kuijper[9]

[1]Department of Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands [2];Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands; Department of Gastroenterology and Hepatology, VU University Medical Center, Amsterdam, The Netherlands; [3]Department of Internal Medicine, Academic Medical Center, Amsterdam, The Netherlands; [4] Department of Internal Medicine and Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands [5]Department of Internal Medicine, Havenziekenhuis, Rotterdam, The Netherlands; [6]Department of Medical Microbiology, VU University Medical Center, Amsterdam, The Netherlands; [7]Dept. of Biobanking and Gastroenterology, Leiden University Medical Center, Leiden, The Netherlands; [8]Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, The Netherlands; Department of Gastroenterology and Hepatology, Haaglanden Medical Center, The Hague, The Netherlands; [9]Department of Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands; European Study Group of C. difficile (ESGCD), European Society of Clinical Microbiology and Infectious Diseases, UK.

Background and aims: Since 2016, the Netherlands Donor Feces Banks is facilitating the treatment of patients with multiple recurrences of Clostridioides difficile infections (rCDI) with fecal microbiota transplantations (FMT).

Methods: An observational study was performed using a standardized approach of data collection and guidance of an expert team of medical microbiologists, infectious diseases physicians and gastroenterologists.

Results: Between March 2016 and March 2018, 260 of 472 donor candidates completed a questionnaire; 221 (85%) were excluded, mainly because age above 50 and an unhealthy BMI. 39 (15%) donor candidates were invited for laboratory screening of blood and feces of which 15 (38%) passed this screening. Carriership of Blastocystis hominis, Dientamoeba fragilis and Multi Drug Resistant Organisms were the most observed exclusion criteria. Of 15 donors, 6 failed at a following screening test, which is performed every two months. Finally, 9 (3.5%) donors were enrolled. Between March 2016 and March 2018, 99 patients were evaluated by our FMT expert team. Of these 99 patients, 23 (23%) were rejected because of underlying bowel disease with *Clostridium difficile* carriership. The mean age of the 76 FMT treated patients was 72 year, 58.6% was female, and the mean recurrence rate was 3.4 CDI episodes. The treatment was performed in 28 different hospitals with a success rate of 89%. 8 patients suffered from CDI relapse, of which 4 were associated with antibiotic use within one month after FMT. All 8 were successfully treated, of which 7 with anti-CDI antibiotics again. 2 serious adverse events of fecal regurgitation were reported without further complications.

P51

Conclusion: Only a low percentage (3.5%) of healthy volunteers is qualified as suitable feces donor. Critical evaluation of FMT applications in a multidisciplinary setting is useful, as a high percentage (23%) of FMT request was rejected due to *Clostridium difficile* carriership instead of infection. An high success rate of 89% for FMT for rCDI was observed. Antibiotic stewardship after FMT is of importance as a high proportion of CDI relapses after FMT is caused by antibiotic use.

P52

RIDINILAZOLE (RDZ) REDUCES RECURRENCE OF *Clostridium difficile* INFECTION (CDI) WITH MINIMAL IMPACT ON THE GUT MICROBIOTA

Vickers Richard[1], Roblin David[1], Duperchy Esther[1], Goldstein Ellie[2], Thorpe Cheleste[3], Snydman David[3], Kane Anne[3], Freeman Jane[4], Wilcox Mark[4].

[1]Summit Therapeutics, Abingdon, United Kingdom; [2]UCLA, Santa Monica, CA;USA; [3]Tufts Med. Sch., Boston, MA, USA; [4]Leeds Teaching Hospital, Leeds, United Kingdom.

Recurrence of CDI (rCDI) is a particular concern with significant impacts on patient welfare and healthcare resources. RDZ is a novel targeted spectrum antibiotic under investigation to treat CDI and reduce rCDI. Here translation of in vitro data to clinical trial data is reviewed.

Susceptibility testing was performed to CLSI standards with vancomycin (VAN), metronidazole (MTZ) and fidaxomicin (FDX) as comparators. The Phase 2 clinical trial was a double-blind, randomised, study of 100 patients assigned 1:1 to 10 days RDZ 200 mg BID or VAN 125 mg QID treatment. Primary endpoint was sustained clinical response (SCR), defined as cure at end of therapy (EOT) and no rCDI for the next 30 days. Primary analysis population was the modified intent-to-treat (MITT); all randomised subjects with a diagnosis confirmed by presence of free toxin. Relative effects of RDZ and VAN on the gut microbiota was examined by sequencing 16S rDNA amplicons from stool collected at baseline, days 5, 10, 25 and end of study. Bioinformatic analyses were performed in QIIME.

Across 4 studies RDZ *Clostridium difficile* (N=439) MIC range was 0.015-0.5µg/mL with no major differences by ribotype or resistance phenotype. RDZ and FDX were less active against Gram negative anaerobes, especially B. fragilis group, than VAN and MTZ. RDZ had limited activity against Gram positive anaerobes. The Phase 2 clinical study exceeded its primary endpoint (MITT), with RDZ shown to be superior on SCR to VAN with rates of 66.7% and 42.4%, respectively. Superiority on SCR was driven by a reduction in rCDI for RDZ (14.3%) compared with VAN (34.8%). Microbiota analysis showed at RDZ EOT that significant relative abundancy reductions were limited to 2 Firmicute families including Peptostreptococcaceae (includes *Clostridium difficile*). In contrast VAN at EOT resulted in significant losses (often to below detection) in 4 Firmicutes families: Peptostreptococcaceae, Ruminococcaceae, Erysipelothrichaceae and Lachnospiraceae. A 70% drop in Actinobacteria, and greater than 3 log decrease in Bacteroidetes, abundance were also observed. These changes were associated with a 25-fold increase in Proteobacteria abundance, in particular Enterobacteriaceae.

These data demonstrate targeted *Clostridium difficile* activity with RDZ both in vitro and in CDI patients which likely resulted in superior SCR compared with VAN. Further clinical development is warranted.

P53

VISUALIZATION OF THE ASSOCIATION OF FIDAXOMICIN AND *Clostridioides difficile* SPORES

Bassères E, Endres B., Rashid T. Alam M. J., Garey K.W.

University of Houston, College of Pharmacy, Houston, Texas, USA

Fidaxomicin has demonstrated novel pharmacologic effects on *Clostridium difficile* spore formation including inhibition of outgrowth of spores and decreasing spore production. However, the association between fidaxomicin and effect of spores has not undergone rigorous microscopic evaluations. The aim of this project was to directly visualize the fidaxomicin-spore association using confocal microscopy. We used a bodipy-fidaxomicin compound with an excitation/emission spectrum equivalent to FITC (461- to 489-nm excitation and 501- to 549-nm emission). The MIC of the fluorescent fidaxomicin was measured in BHIS using the serial dilution method. Spores were harvested after 5 days incubation in anaerobic chamber on blood agar plates at 37°C, purified on 50% sucrose gradient and standardized at ≈107CFU/mL. One hundred µl of spores and 100 µg/mL bodipy-fidaxomicin were incubated together for 1h at room temperature in PBS, washed 5 times, and the final pellet was re-suspended in water. The samples were stained with FM4-64 to visualize the membrane and analyzed on a Leica SP8 confocal microscope using a 63x objective. The MIC of the labeled fidaxomicin was 1µg/mL compared to 0.06µg/mL for the non-labeled fidaxomicin. Intra-cellular accumulation of bodipy-fidaxomicin inside vegetative cells was previously observed. Interaction of spores with the fluorescent fidaxomicin was assessed against 3 different ribotypes: 027; 012 and 078. Bodipy-fidaxomicin surrounding the *Clostridium difficile* spores was observed for all ribotypes. In conclusion, this study showed a direct association between fidaxomicin and *Clostridium difficile* spores providing advanced insight regarding the anti-spore properties of this compound.

P54

ISOLATION AND CHARATERISATION OF A NOVEL BACTERIOCIN THAT IS ACTIVE AGAINST CLINICALLY RELAVENT STRAINS OF *Clostridium difficile*

Sarah Chapman[1], Michelle Kelly[1], Sarah Kuehne[2], Alan Cockayne[1], Christopher Penfold[3]

[1] University of Nottingham, Synthetic Biology Research Centre, Centre for Biomedical Sciences, Faculty of life Science, Nottingham, UK; [2] University of Birmingham, School of Dentistry, Birmingham, UK; [3] University of Nottingham, Faculty of Medicine & Health Sciences, Nottingham, UK

Background

Current treatments for *Clostridium difficile* Infections (CDI) are under threat due to antibiotic resistance thus a novel therapy is essential. A major risk factor for CDI is the use of broad-spectrum antibiotics therefore different therapies need to be explored. Bacteriocins are bacterial antimicrobial peptides that are active against other bacteria strains. Environmental strains could be a reservoir for bacteriocins and thus result in a novel CDI therapy.

Method

Bacteria isolated from cow manure were screened for activity against *Clostridium difficile* strains via an overlay. An isolate of interest showed a zone of clearance against at least two *Clostridium difficile* strains and was identified using 16S sequencing and sent for whole genome sequencing. Sterile filtered spent supernatant of the isolate of interest was inoculated with exponentially growing *Clostridium difficile* to identify activity from a potentially secreted protein and this was repeated with precipitated supernatant proteins. The same precipitant was also run on a tricine SDS-page gel overlaid with inoculated agar to try and correspond a zone of clearance with a band of protein.

Results

A Bacillus pumilus strain was isolated which showed activity against *Clostridium difficile* R20291, DH1916, CD630, and 5 other strains. The spent supernatant of the B. pumllus strain inhibited *Clostridium difficile* CD630, DH1916 and CD196 and the corresponding supernatant precipitated proteins showed activity against *Clostridium difficile* CD630, and further strains require testing. The precipitant also showed two zones of clearance on the SDS-page gel, corresponding to silver stained bands. The whole genome sequence showed one potential bacteriocin, a class V cyclical bacteriocin and a predicted bacteriocin self-immunity gene. A class phylogenetic tree showed that the sequence clusters with Circularin A from C. beijerinckii. The predicted molecular weight and actual molecular weight of Circularin A is comparable to the predicted molecular weight of the potential bacteriocin and a zone of clearing on the SDS-page gel.

Conclusions

There is evidence that the isolated B. pumilus strain is secreting a proteinous molecule that is active against multiple *Clostridium difficile* strains. The presence of a potential bacteriocin in the genome suggests this could be responsible for the observed activity however, further investigation is required to show a directly link.

P55

ABSENCE OF PASSIVE TRANSFER OF TOXIN GENES FROM TOXIGENIC *Clostridium difficile* (CD) TO NON-TOXIGENIC *Clostridium difficile* (NTCD) STRAIN M3

Susan P. Sambol[1,2], Stuart Johnson[1,2], Dale N. Gerding[1,2]

[1]Edward Hines, Jr. VA Hospital, Hines, IL [2]Loyola University Chicago Stritch School of Medicine, Maywood, IL

NTCD strain M3 is a Restriction Endonuclease Analysis (REA) type that protects colonized hamsters from *Clostridium difficile* infection (CDI) when challenged with toxigenic CD, and prevents recurrent CDI in colonized patients. Recent studies have shown transfer of the Pathogenicity Locus (PaLoc) from toxigenic CD strain 630 (REA Group R) to NTCD strain CD37 (REA Type T18) by conjugation, creating toxin-producing mutants of the NTCD strain. This caused concern that passive transfer of PaLoc genes could potentially compromise the protective effect of NTCD colonization.

To compare the efficiency of PaLoc passive transfer, we replicated the previous experiments using strain 630ΔtcdA as donor and CD37 as recipient, and repeated the experiment using M3 as the recipient strain. 630ΔtcdA has an ermB gene inserted into its tcdA gene but is rifampicin susceptible, whereas CD37 is rifampicin-resistant. We generated a M3 strain resistant to rifampicin at 50 ug/ml by repeated passage on rifampicin-containing plates. Both CD37 and M3 strains are erythromycin-susceptible and become erythromycin resistant if they acquire the ermB gene. Overnight broth cultures of donor and recipient strains were combined in 1:1 proportions on nitrocellulose filters on BHI agar with 5% horse blood. The matings were incubated for 24 hours, then each filter washed with 2 ml of sterile BHI, and the filtrate inoculated onto 20 selective plates containing rifampicin (25 ug/ml) and erythromycin (10 ug/ml). Plates were incubated for 96 hours, and colonies from each mating were analyzed by REA typing and by PCR for tcdB genes.

The 630ΔtcdA/ CD37 mating produced 12 passive transfer colonies in 10^9 recipient cfus, similar to the frequency previously reported (7.5 per 10^9 cfus). Eleven of the 12 colonies showed minor changes in REA pattern from parent CD37/T18 strain; only one colony was identical to T18. Regardless of changes in REA type, all passive transfer colonies showed the presence of tcdB gene.

The 630ΔtcdA/M3 matings in duplicate produced no passive transfer colonies in 3.5 x 10^9 M3 recipient cfus.

These results suggest REA type M3 is less likely to acquire toxin genes compared to strain CD37 but will require repeated confirmatory mating experiments. The mechanism of transfer inhibition is the subject of ongoing investigation.

The authors thank Peter Mullany and Sarah Kuehne for their invaluable assistance.

P56

EVALUATING SAFETY AND EFFICACY OF MICROBIOTA-BASED THERAPIES FOR RECURRENT *Clostridium difficile* INFECTION: THE ADDED INSIGHT OF MICROBIOME PROFILING AND THE MICROBIOME HEALTH INDEX IN TWO PHASE 2 CONTROLLED TRIALS OF RBX2660

Ken Blount[1], Robert Orenstein[2], Erik Dubberke[3], Sahil Khanna[4], Gail Hecht[5], Sarah Mische[1]

[1]Rebiotix, Inc. Roseville, MN; [2]Division of Infectious Diseases, Mayo Clinic in Arizona, Phoenix, AZ, USA; [3]Department of Internal Medicine, Washington University of St. Louis, St. Louis, MO; [4]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA; [5]Division of Gastroenterology and Nutrition, Loyola University Medical Center, Chicago, IL, USA

Background and Aims

Numerous microbiota-based therapies are being evaluated to treat recurrent *Clostridium difficile* infections (rCDI). RBX2660, a standardized, stabilized microbiota-based investigational drug, has demonstrated efficacy for preventing rCDI in two multicenter Phase 2 controlled trials. Participant microbiome analysis suggests that RBX2660 restores a healthier microbiome composition. Here we present clinical and microbiome results, including the development of microbiome biomarkers.

Methods: A randomized, double-blinded Phase 2B trial (PUNCH CD2) compared the efficacy of one and two doses of RBX2660 to placebo. An open-label study (PUNCH OL) compared the efficacy of RBX2660 to that of standard-of-care antibiotics via a historical control cohort. For both studies, success was defined as absence of CDI recurrence through 8 weeks after the last treatment. Participant fecal samples from before, 7, 30, and 60 days after treatment were sequenced using either 16S or a shallow shotgun method to facilitate microbiome comparisons. The resulting taxonomic distributions were used to generate a prototype Microbiome Health Index™(MHI) as a potential unidimensional microbiome biomarker.

Results: In PUNCH CD2, the efficacy among participants who received RBX2660 was 67%(n=83) compared to 46% for placebo-treated subjects(n=42; p=.046). In PUNCH OL, RBX2660 efficacy was 79%(n=136), compared to 52% in the Control group(n=110; p<.0001). Adverse events(AEs) and serious AEs were not significantly different between groups in both trials. For both trials, RBX2660 treatment shifted participant microbiome compositions, with increases in Bacteroidia and Clostridia and decreases in Gammaproteobacteria and Bacilli. Combining the microbiome data across trials, a putative diagnostic threshold MHI was determined by comparing pretreatment to healthier microbiomes. As early as 7 days post-treatment, the MHI threshold could distinguish between successful or failed RBX2660 treatment(p=.003).

Conclusion: These controlled Phase 2 trials demonstrate RBX2660 safety and RBX2660 efficacy in preventing rCDI when compared to placebo-treated and historical control groups. Furthermore, evaluation of participants' microbiome during and after treatment provides greater understanding of rCDI prevention and informs microbiota-based therapy development. This analysis was funded by Rebiotix, Inc.(Roseville,MN).

P57

ORAL TEICOPLANIN FOR SUCCESSFUL TREATMENT OF RECURRENT *Clostridium difficile* INFECTION

Nikolic Natasa[1,2], Korac Milos[1,2], Nesic Zorica[3], Markovic Aleksandar[1], Mitrovic Nikola[1,2], Milosevic Ivana[1,2]

[1] University Hospital for Infectious and Tropical Diseases, Clinical Center Serbia, Belgrade, Serbia
[2] Medical Faculty, University of Belgrade, Belgrade, Serbia
[3] Department for pharmacology, clinical pharmacology and toxicology, University of Belgrade, Belgrade, Serbia

Background and aims: *Clostridium difficile* is the major cause of hospital-acquired diarrhoea and one of the most frequent causes of nosocomial infections in general. High recurrence rate, especially after second episode of the disease, which can be as high as 45-60%, is one of most important issues in everyday practice with *Clostridium difficile* infection (CDI).

Method: We performed a prospective observational study in which we compared the efficacy of teicoplanin and vancomycin in the treatment of patients with first recurrence of severe or complicated CDI concerning clinical cure and recurrence rate.

Results: We analysed 101 patients who suffered their first recurrence of CDI and had severe or complicated form of the disease. Among them 36 were treated with oral teicoplanin 100mg bid, and 65 were treated with oral vancomycin 125mg bid. The mean age of patients was 76.2 $\pm$10.6 years, and there was no statistically significant difference in the mean age of patients treated with vancomycin and teicoplanin (p 0,723). Three patients treated with teicoplanin (8.3%) and 7 (10.7%) treated with vancomycin had complicated CDI. There was no statistically significant difference in clinical cure rates among patients treated with teicoplanin and vancomycin (83.3% vs 84.6%, p=1,000). Eight (1.1%) patients treated with vancomycin and 3 (8.3%) treated with teicoplanin died. After eight weeks of follow up, 9% of patients treated with teicoplanin and 30.9% of those treated with vancomycin suffered another relapse. Recurrence rate achieved after teicoplanin treatment was statistically significantly lower in comparison to the one achieved after vancomycin treatment (p=0.020).

Conclusions: Teicoplanin might be a good treatment option for patients with severe and severe complicated CDI with low recurrence rates.

P58

HIGH RATE OF MULTI DRUG RESISTANT CLADE 4 *Clostridium difficile* ISOLATES FROM CHINA AND CARRIAGE OF ANTI BIOTIC RESISTANT GENES

Yuan Wu, Wen-zhu Zhang, Wen-ge Li, Jin-xing Lu

State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China;

Background and aims: To investigate the antibiotic resistance profile and possible antibiotic resistant genes of clade 4 *Clostridium difficile* isolates from China.

Methods: Thirty-seven clinical *Clostridium difficile* isolates of clade 4 from China, including ST37, ST81, ST39, ST109, and ST332, were included for antibiotic analysis. *Clostridium difficile* isolates were tested for susceptibility to moxifloxacin (MXF), vancomycin (VAN), clindamycin (CLI), tetracycline (TET), erythromycin (ERY), rifampin (RFX), levofloxacin (LFX), chloramphenicol (CHL), metronidazole (MTZ), ciprofloxacin (CIP), and meropenem using E-test strips (Biomerieux, France, and Liofilchem, Italy). The interpretation of minimum inhibitory concentration (MIC) for MTZ, MXF, CLI, CIP, LFX, and TET were done according to recommendations of CLSI M11-A7 and M100-S23. The breakpoints for VAN, RFX, ERY, CHL, and meropenem were determined following a previous study. Multidrug resistant (MDR) were defined as resistance to at least three antimicrobial classes. *Clostridium difficile* ATCC 700057 was included as a control in each experiment. Antibiotic resistance genes were predicted by comparing with CARD and ARDB database using WGS.

Resultes: Except for 10122, all the other 36 isolates are MDR strains, accounting for 97.30%. For quinotone, resistant to CIF was as high as 100%, followed by LFX (97.30%) and MXF (56.76%). Resistance to ERY stayed at a high level(91.89%). Twenty-five out of 34 ERY-resistant isolates were detected with gene ermB (73.53%). The resistant rate to CLI is 89.19% while resistance to CHL stayed in a lower rate (29.73%), and related genes cata11 and cata1 were only identified in isolate ZR18 and M68, respectively. What deserves to be mentioned is that gene catD, normally carried by Tn4453a/b and also responsible for CHL resistance, was present in isolate ZR18, which showed middle-level of CHL resistance (MIC=64 μg/ml); but was replaced by another five genes of isolates 7, 2, 28, which is CHL susceptible. Within the 5 new replaced genes, aac (6')aph(2') was included, which is significantly correlated with HLGR and responsible for aminoglycoside resistance. MEM (51.35%) and RFX (48.65%) showed close resistant rate. . Genes rpoB and rphB reported as determinants for RFX resistance, were detected in almost all isolates.No isolates was found resistant to VAN and MET, although VAN related genes or gene cassette were identified.

Conclusions: The MDR isolates and the resitant rate these drugs increased obviouly compared with other studies in China. However, they are still sensitive to VAN and MET. The mechanism of resistance is complex, not only because of the presence of specific genes, but also of unique substitution in specific genes. Moreover, (MGEs) play an important role in obtaining new antibiotic resistant genes from local microenviroment.

P59

ABSENCE OF A METRONIDAZOLE RESISTANCE MARKER IN *Clostridioides difficile* PCR-RIBOTYPE 078 ISOLATES FROM PORCINE ORIGIN

Eloisa Sevilla[2], Celine Harmanus[1], Ilse Boekhoud[1], Bastian Hornung[1], Raúl Carlos Mainar-Jaime[2], Rosa Bolea[2], Wiep Klaas Smits[1], Ed Kuijper[1]

[1]Department of Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands; [2]Departamento de Patología Animal, Facultad de Veterinaria, Instituto Agroalimentario de Aragón – IA2 (Universidad de Zaragoza-CITA), Zaragoza, Spain

Metronidazole is currently used as a first-line agent in the treatment of Clostridioides difficile infection (CDI) in humans and animals. However, reduced susceptibility to this antimicrobial agent has emerged in *Clostridium difficile* and several mechanisms have been proposed to be involved. Recently, we identified a plasmid in non-toxigenic human and animal *Clostridium difficile* ribotype 010 strains with a high level of metronidazole resistance that is absent from susceptible isolates. Its presence in other ribotypes is still unknown. Apart from that, food-producing animals are regarded as a potential reservoir of *Clostridium difficile*, and may also harbor multidrug resistant strains. Even though foodborne transmission of this pathogen is not yet established, the role of animals in human CDI and in dissemination of antimicrobial resistant strains should be of concern. The aim of this study was to evaluate the presence of pCD-metro in *Clostridium difficile* strains belonging to the hypervirulent PCR ribotype 078 and isolated in pig farms.

A total of 394 *Clostridium difficile* strains were analyzed in this study. All isolates were identified as PCR ribotype 078 by capillary ribotyping. All strains were isolated from either animal (67.8%) or environmental (32.2%) samples collected in The Netherlands and Spain between 2009 and 2017. Metronidazole susceptibility testing was performed according to the CLSI recommended agar dilution method using the breakpoint defined by EUCAST (ECOFF >2 mg/L). Bacterial DNA was extracted from pure cultures using QIAamp Mini Spin Columns (Qiagen). The detection of the plasmid was performed by conventional PCR using DNA from a characterized plasmid-positive strain as a control.

The MIC50/MIC90 for metronidazole for the 82 isolates tested by agar dilution was 0.25/0.25 mg/L and no resistance was observed. None of the 394 isolates tested by PCR was positive for the marker.

Our data show that the metronidazole resistance marker, and metronidazole resistance, is not common among PCR-ribotype 078 *Clostridium difficile* isolates from porcine origin. Nevertheless, surveillance of metronidazole susceptibility and resistance mechanisms is needed in order to improve the management of CDI.

P60

FLUOROQUINOLONE RESISTANCE MUTATION CONFERS A FITNESS ADVANTAGE ON *Clostridium difficile* IN A CONTINUOUS CO-CULTURE MODEL

Jonathan Vernon [1], *Mark Wilcox* [1,2], *Jane Freeman* [1,2]

[1]*Healthcare-Associated Infections Research Group, Molecular Gastroenterology, Leeds Institute for Biomedical and Clinical Sciences, University of Leeds, United Kingdom.* [2] *Leeds Teaching Hospitals Trust, Leeds, United Kingdom.*

Background: The link between fluoroquinolones (FQs) and *Clostridium difficile* infection is well known. FQ resistance was implicated as a driver in large *Clostridium difficile* PCR ribotype 027 (RT027) outbreaks in the early 2000s and is common amongst prevalent PCR RTs However, the relevance of FQ resistant mutations and their impact on bacterial fitness (BF) is less clear.

Methods: BF of 7 RT 027 mutant isolates in gyrA (Thr82-Ile n=4; Asp71-Tyr n=1) or gyrB (Gly429-Val; Gln434-Lys) with reduced susceptibility to moxifloxacin (MXFR)(4-32 mg/L) were compared to their susceptible (<2 mg/L) progenitor strains in competitive batch culture, cell cytotoxicity and maximal growth rate assays. The comparative BF dynamics of a Thr82-Ile (gyrA) harbouring isolate, CD3079M vs parent strain (CD3079) were also investigated in a continuous co-culture (CC) chemostat model. Mutant and parent strain populations were assessed every 24hrs over 8 days using selective and non-selective agars. Parent BF was set at w=1. Further resistance development was monitored with 32 and 64 mg/L MXF-incorporated agars. Sequencing was achieved using NEBNext® Ultra™ chemistry and Illumina®HiSeq3000 technologies.

Results: BF was significantly increased in all Thr82-Ile exhibiting isolates (w=1.08-1.22) in competitive batch culture assays; (p=0.002). Gly429-Val and Gln434-Lys, also showed a BF advantage (w=1.24 and 1.18, respectively), but, Asp71-Tyr conferred a burden (w=0.80). CC results for strains CD3079 and CD3079M (Thr82-Ile) supported batch culture results; mutant to parent ratios differed significantly by 96 hours ($\bar{x}\,\bar{x}$ =1.80; p=0.025). MXFR mutant BF remained relatively consistent for the duration of the CC model (w=1.11–1.45, $\bar{x}$ =1.25), in close support of batch findings (w=1.15). Mutants with further elevated MXFR (64 and 128 mg/L) were generated within the first 24hrs, 7.34×10^4 and 4.44×10^1 log10CFU/ml, respectively, although these high level mutants rapidly became undetectable. Consistent trends were observed across 3 CC model replicates.

Conclusions: The absence of a BF cost associated with the most prevalent FQ resistance mutations may have contributed to the success of the RT 027. Furthermore, a demonstrable in vitro advantage over FQ sensitive parent strains in CC may be a contributory factor to the maintenance of RT 027, even in the absence of FQ pressure.

P61

ANTIMICROBIAL RESISTANCE OF *Clostridium difficile* ISOLATES OBTAINED FROM TWO PIG FARMS IN LONGITUDINAL STUDY

Pirš Tina, Matjaž Ocepek, Avberšek Jana

University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia

Clostridium difficile is an important pathogen for humans and animals and there is a concern about the possibility that food animals, including pigs, might serve as a reservoir of epidemic strains. The aim of our work was to determine antimicrobial susceptibility of the pig isolates of *Clostridium difficile*. The isolates were obtained in the longitudinal study in intensive/semi-intensive farms where we collected isolates starting with breeding sows before giving birth, then sampling the offspring up to 170 days and breeding sows again in the new cycle. The samples of dust were also included. All pig isolates belonged to PCR-ribotypes 078, 045 and 150.

MIC values for a total of 132 *Clostridium difficile* isolates from two pig farms were determined with a customized broth microdilution plate (Micronaut S CD MIC) (Merlin Diagnostika GmbH, Germany). Seventeen antimicrobials were selected, including amoxicillin, ceftriaxone, clindamycin, daptomycin, erythromycin, fusidic acid, imipenem, levofloxacin, linezolid, metronidazole, moxifloxacin, oxacillin, rifampicin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole and vancomycin. The ermB and tetM genes were detected with PCR.

All tested strains were susceptible to metronidazole and vancomycin. One isolate that was detected as metronidazole resistant with this method was later excluded as resistant with E-test. The ermB gene was detected in 59.1 % isolates of PCR-ribotype 150, which correlated with high rate of resistance to erythromycin in these strains ($\geq$512 µg/ml). 80 % of those strains were also resistant to clindamycin ($\geq$256 µg/ml). In general, tetracycline displayed low MICs values and only one isolate was resistant. Three isolates of PCR-ribotype 150 were resistant to moxifloxacin. The tetM gene was mostly detected among PCR-ribotype 045. However, all tetM positive isolates were phenotypically susceptible to tetracycline with MIC values of 4 µg/ml. Resistance rates for daptomycin and oxacillin were different between PCR-ribotypes 045/078 (toxinotype V) and 150 (toxinotype 0).

The results demonstrated multidrug resistant strains among isolates. However, the resistance patterns were typical for the PCR-ribotype and have not changed during the longitudinal study within the farm. Our results add further evidence about food animals being the possible reservoir of antimicrobial resistance determinants.

MOLECULAR DIAGNOSTICS IN CDI: SWITCHING TO NAAT AS SCREENING METHODOLOGY?

P62

Keith Chiasson, Nancy Paquette, Jeroen I. Stas, Patrice Allibert[1].

[1]GenePOC Inc., Québec, Canada

Background: Despite more than a decade of dedicated research on standardization of guidelines, *Clostridium difficile* infections (CDI) still create a tremendous economic burden while complicating proper patient management. The current European guidelines recommend the use of a two-step algorithm with an initial screening test followed by a confirmation test of the positive results. Similar recommendations were recently put in place by the Infectious Disease society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) for commonly submitted stool specimens for which no pre-agreed institutional criteria are defined. However, if stool specimens are derived from CDI-likely patients using pre-agreed institutional criteria, use of standalone nucleic acid amplification techniques (NAAT) is allowed. Here we report the performance data of the GenePOCTM CDiff assay obtained from two separate multicenter evaluations; one in the US and one in Europe.

Materials/Methods: Consecutive diarrheal stool samples from hospitalized patients were assessed between January and December 2017. All assays (GDH, Toxin A&B, NAAT) were performed in alignment with the manufacturer's instructions. Direct toxigenic culture was performed as golden standard method.

Results: Combined, 5080 stool specimens were evaluated in both the US (2461) and Europe (2619). Overall, the GenePOC™ CDiff assay displayed a good valid result calling with only 2.1% and 4.1% invalid results for the US and Europe, respectively. In total, only 4 samples (< 0.1%) remained invalid after repeat testing. In comparison to direct culture performed on fresh stool specimens the sensitivity and specificity (with respective 95% CI) were: 95.5% [87.3 – 99.1] and 93.4% [91.4 – 95.1]. When compared to the routine CDI diagnostics, standalone GenePOC™ CDiff testing displayed an excellent agreement as scored by a kappa value of 0.95.

Conclusions: In accordance to both the European and the IDSA/SHEA guidelines, the overall performance of the GenePOC™ CDiff assay makes it a suitable testing platform that either could be used as screening test or as standalone method.

P63

DETECTION OF TOXIN-PRODUCING *Clostridium difficile* IN THE PAEDIATRIC POPULATION AT GREAT ORMOND STREET HOSPITAL (GOSH): AN EVALUATION OF CURRENT METHODOLOGIES AND DEVELOPMENT OF A TESTING ALGORITHM

Gintare Vaitkute[1], Francis Yongblah[2], Tanja Rockenbach[2], Mona Bajaj-Elliott[1], John Hartley[2] and Elaine Cloutman-Green[2]

[1] University College London, Great Ormond Street Institute of Child Health 30 Guilford Street, London UK, [2] Great Ormond Street Hospital, London, UK.

Background and Aims: Diagnosis of *Clostridium difficile* infection versus carriage is problematic within the paediatric population, especially when diarrhoea is present. Due to cessation of the use of the cell cytotoxicity neutralisation assay (CCNA) for diagnosis; available alternatives (toxin gene PCR and an enzyme immunoassay (EIA) for toxin and glutamate dehydrogenase) were evaluated and their position within a diagnostic testing pathway assessed.

Methods: 181 stool samples were utilised to assess the performance of the Quik Chek Complete (QCC) EIA assay and two real-time TcdA and TcdB PCR assays (artus *Clostridium difficile* QS-RGQ MDx, Qiagen; Realstar *Clostridium difficile*, Altona) independently or in a combined algorithm (Algorithm 1- Qiagen PCR+ QCC; Algorithm 2- Altona PCR+QCC). Performance was evaluated against the CCNA assay.

Results: Sensitivity, specificity, positive and negative predictive values were as follows. QCC assay- 85.3%, 100%, 100%, 91.9%; QCC assay (GDH antigen only)- 100%, 75.2%, 67.4%, 100%; Qiagen PCR- 100%, 91.2%, 87.2%, 100%; Altona PCR 100%, 88.5%, 84%, 100%; Algorithms 1 and 2- 85.3%, 100%, 100%, 91.9%. Conclusions: No individual test was superior to CCNA assay. The 2-step algorithm, which uses PCR followed by the QCC assay evaluated best and has thus been adopted for routine use. This allows the detection of toxin-production capable *Clostridium difficile*, while also assessing whether the organism is currently producing toxin to enable infection control risk assessment. PCR followed by EIA can be undertaken within 48 hours, if required to meet the mandated UK timeframe.

P64

ENHANCED SENSITIVITY AND ACCURACY BY LASER READING OF RAPID TESTS FOR *Clostridium difficile* GDH DETECTION

Van Broeck Johan, Ochoa Theysa, Soumillion Kate, Ngyuvula Eléonore and Delmée Michel

Belgian Reference Centre for Clostridium difficile, Pôle de microbiologie médicale, Université Catholique de Louvain, Brussels, Belgium

Introduction: Glutamatedehydrogenase (GDH) screening with toxin detection followed by a nucleic acid amplification test (NAAT) is accepted by the European guidelines as a good two or three step algorithm for the detection of toxigenic *Clostridium difficile* in stool. Rapid immunological tests give often problems in arbitrary reading. Here we evaluated the Clostridium K-SeT GDH stool test using two different laserscan tools which we compared to visual reading by two independent persons.

Methods: Stools were collected from inpatients at the University Hospital St-Luc - UCL suffering from diarrhea. Between March 2018 and April 2018, 206 stools were tested for GDH using the Liaison® *Clostridium difficile* GDH assay (Diasorin, Stillwater, USA), the Quik Chek Complete (Techlab® Blacksburg, USA) and the Clostridium K-SeT (Coris BioConcept, Gembloux, Belgium). Cultures were performed on ChromID® C. diff (bioMérieux). NAAT was performed using the C.difficile LIAISON® MDX. The rapid GDH test was read visually by two different persons and two different laser scanners the aLF (Qiagen, Hilden, Germany) and the Skan-Smart (Skannex, Oslo, Norway).

Results:Visual reading by two persons gave discordant results in 3.9%. Liaison GDH gave a sensitivity of 96.3%. Laserscan reading with Skansmart enhanced sensitivity for GDH from 88.6% to 95.5% towards visual reading. Laserscan reading with the aLF enhanced sensitivity for GDH from 88.6% to 90.7% towards visual reading. Skansmart reading generated less false negative GDH samples (N=2) than aLF reading (N=4) or visual reading (N=5).

Discussion and conclusion: Laserscan reading gives not only an objective traceable reading but it enhances sensitivity and accuracy of GDH detection in the Coris BioConcept rapid Clostridium K-SeT.

P65

GDH AND TOXIN A/B CONCENTRATIONS DO NOT DEPEND ON STOOL CONSISTENCY IN PATIENTS WITH CLINICAL SUSPICION FOR CDI

Koskinen Juha M.[1,2], Savolainen Roosa[3], Koskinen Janne O.[2], Kaukoranta Suvi-Sirkku[3]

[1]Turku Doctoral Programme of Molecular Medicine, University of Turku, Turku, Finland; [2]ArcDia International Ltd, Turku, Finland; [3]Department of Clinical Microbiology, Vaasa Central Hospital, Vaasa, Finland

ESCMID diagnostic guidance for *Clostridium difficile* infection (CDI) emphasizes clinical signs and symptoms as the most fundamental criteria for CDI diagnosis. The guideline recommends not to test formed stools for CDI except when a patient has paralytic ileus. Recent studies suggest that toxin A/B positivity predicts CDI. Furthermore, quantification of glutamate dehydrogenase (GDH) and toxins A/B may have diagnostic value by and aiding in prognosis and assessing therapy.

mariPOC® CDI (ArcDia Int. Ltd, Finland) is a new automated antigen test that analyses *Clostridium difficile* GDH and toxin A/B from stool samples. The test can provide quantitative numerical information about the analyte concentration in the stool. This is shown as signal strength value psi (Ψ). The scale for the psi value is analyte dependent and the quantification power between patients and consecutive samples is limited by physiological conditions and the analyte secretion.

The purpose of this study was to evaluate the usefulness of the new semi-quantitative test as a tool for CDI diagnostic studies. The stools (N=331) were collected from symptomatic patients and tested, as part of routine, for *Clostridium difficile* toxin B gene by PCR (Abacus Diagnostica Oy, Finland) in Vaasa Central Hospital, Finland, during May to September 2017. Median age was 74 (13□98) years. The samples were analysed with mariPOC CDI test, membrane enzyme immunoassay, PCR and culture methods. The data was analysed with respect to correlation between stool consistency (solid, loose, watery) and semi-quantitative detection of GDH and toxins.

There were 38 GDH and 30 toxin A/B positive mariPOC results confirmed by other tests. High GDH and toxin A/B concentrations were detected irrespectively of the consistency of the stool. Statistical differences were not observed between different stool consistencies and analyte concentrations (t-test, all >0.1). The highest toxin concentrations were estimated to be at least 0.3 micrograms per gram of stool. Interestingly, the two watery samples that had high toxin concentrations had only low or moderate concentration of GDH.

Our results encourage further studies to assess the diagnostic value of GHD and toxin A/B quantification and the significance of stool consistency in CDI management. The new mariPOC CDI test provides a practical tool not only for diagnostic testing but also for scientific studies.

P66

THE SIMOA ASSAY FOR DETECTION OF *Clostridium difficile* TOXINS HAS A BETTER SENSITIVITY THAN THE CYTOTOXICITY ASSAY.

Gateau Cécile [1], Gouot Cyril [1], Lapidus Nathanael [2], Couturier Jeanne [1,3,5], Lalande Valérie [3], Foussadier Agnes4[1], Banz Alice [4], Barbut Frédéric [1,3,5].

[1]National Reference Laboratory for Clostridium difficile, Saint-Antoine Hospital, AP-HP, Paris France; [2]Department of Public Health, Saint-Antoine Hospital, AP-HP, Paris France; [3]Department of Microbiology, Saint-Antoine hospital, AP-HP, Paris, France;, [4]bioMérieux, Marcy l'Etoile, France; [5]EA 4065, University Paris Descartes.

Background: *Clostridium difficile* is major agent responsible for healthcare associated diarrhoea. The European guidelines recommend the use of a two-step algorithm for the diagnosis of *Clostridium difficile* infection (CDI) based on a sensitive screening method (GDH detection or NAAT) followed by a more specific test detecting toxins. Commercial EIA tests for detection of toxins display a suboptimal sensitivity and currently cannot be used as standalone test. An ultrasensitive assay detecting free toxins A and B has been recently developed by bioMérieux using the single molecule array technology (SIMOA).

Materials and methods: We compared the sensitivity and specificity of the assay using 100 frozen stools of patients previously diagnosed with CDI by toxigenic culture. Among these patients, 67 had a positive cytotoxicity assay (CTA) on MRC-5 cell culture and 33 a negative CTA. We also tested stools of 38 patients negative for *Clostridium difficile* by culture and 32 patients harbouring a non-toxigenic strain of *Clostridium difficile*. The threshold of positive result with SIMOA was set up at 22 and 18.8 pg/ml, for toxins A and B detection, respectively.

Results: Among the 67 patients with a positive CTA assay, only 3 (4.5%) were negative for both toxins A and B by SIMOA. The remaining stool samples were positive for both toxins (n=59) or for toxin B only (n=5). Among the 33 patients with CDI but negative for CTA, 9 (27.3%) were positive for both toxins by SIMOA, 5 (15.1%) were only positive for toxin A and 2 (6.1%) were only positive for toxin B. Among the 38 patients negative for *Clostridium difficile* by culture, 2 (5.2%) were positive for toxin A only with a very low titre (43 and 40 pg/ml). The 32 patients harbouring a non-toxigenic strain of CDI were all negative for both toxins. Toxins A and B concentrations determined by SIMOA were significantly correlated (Pearson correlation=0.89, p<0.001). Correlations between toxin concentrations, severity of CDI and 30-day mortality are in progress.

Discussion-Conclusion: This study confirmed that the sensitivity of the SIMOA assay is higher than the cytotoxicity assay and can detect faecal toxins in 48.5 % of samples found to be negative using the CTA which is currently the reference technique for detecting free toxins from stools. *Clostridium difficile* toxin detection using SIMOA technology has the potential to improve and simplify the CDI diagnosis.

P67

SUBTYPING *Clostridium difficile* PCR-RIBOTYPE 018 STRAINS BY ANALYSIS OF VIRULOME, RESISTOME, WGMLST AND MLVA

Gateau Cécile [1], Couturier Jeanne [1,3], Barbut Frédéric [1,2,3].

[1]National Reference Laboratory for Clostridium difficile, Saint-Antoine Hospital, AP-HP, Paris France;[2] Department of Microbiology, Saint-Antoine hospital, AP-HP, Paris, France; [3]EA 4065, University Paris Descartes.

Background: *Clostridium difficile* PCR-Ribotype (RT) 018 is an emerging RT associated to severe infections and outbreaks with a transmission index 10-fold higher than that of RT078 strains. As for other common RT i.e 001, 027 or 014, we need more discriminant methods to subtype some specific RT to better understand transmission mechanisms or to investigate outbreaks. We compared MLVA (Multi-Locus VNTR [Variable Number Tandem Repeat] Analysis), wgMLST (whole genome Multi Locus Sequence Typing), virulome and resistome for subtyping RT018 strains.

Materials and methods: A total of 31 RT018 strains including 19 strains from a well-documented outbreak in a geriatric unit (GU) in Strasbourg, France, and 12 epidemiologically unrelated strains from other French healthcare facilities (HCF) were characterized by their virulome and resistome using BIOMERIEUX EPISEQ® CS Software. Strains were subtyped by MLVA and wgMLST. For MLVA typing, seven tandem repeat loci were amplified by PCR. The genetic relationship between two strains was assessed by calculating the summed tandem repeat differences (STRD). Strains with an STRD $\leq$ 10 were defined as genetically related and clonal complexes (CC) were defined by an STRD $\leq$ 2. The data for wgMLST were analysed with BioNumerics® 7.6.3 software. The genetic relationship between two strains was assessed by calculating the number of different alleles. Strains with an allele difference $\leq$ 200 were defined as genetically related and clonal complexes were defined by an allele difference $\leq$ 20.

Results: The MLVA analysis indicated that among the 31 RT018 strains, 19 were included in 2 CC. The first one comprised 9 strains (53%), all isolated in patients from the GU and the second one included 10 strains (6 strains (35%) from the GU and 4 strains (33%) from other HCF). Two strains from GU did not belong to the CC. Analysis of wgMLST resulted in one CC that included 19 strains from GU (100%) and 4 strains (33%) from other HCF. Among the strains from GU 84.2% (16/19) displayed the same resistome whereas 33.3% (4/12) only of the non-epidemic strains had the same epidemic resistome pattern. Virulome was not relevant for discriminating epidemic and non-epidemic 018 strains.

Discussion-Conclusion: MLVA and wgMLST gave quite consistent information but the wgMLST better separated epidemic from non-epidemic strains.

P68

DETECTION OF *Clostridium difficile* BASED ON REAL-TIME PCR WITH PNA PROBE

Jong Wan Kim, Bun Seung Jo, Ha-Young Kim, Chung-Hyun Kim, Yu-Ran Lee, ByungJae So

Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea

Background and aims: *Clostridium difficile* (*Clostridium difficile*) is a spore-forming bacterium that causes enteric disease in different animal species and humans. Pathogenic *Clostridium difficile* strains produce multiple toxins. Toxins A (TcdA) and B (TcdB) mediate the pathogenesis of *Clostridium difficile* infection (CDI), and toxin detection is critical for diagnosis. Most pathogenic strains are toxin A-positive, toxin B-positive (A+B+) strains, although toxin A-negative, toxin B-positive (A-B+) variant isolates have been recognized as pathogenic. The purpose of this study was to develop a multiplex Real-time PCR method using primers, fluorescently labeled peptide nucleic acid (PNA) probe for simultaneous identification and toxigenic type characterization of *Clostridium difficile* isolates.

Methods: The multiplex Real-time PCR detected a species-specific internal fragment of the triose phosphate isomerase (tpi) housekeeping gene, an internal fragment of the toxin A gene (tcdA), and of the toxin B gene (tcdB). Bacterial DNA was extracted from suspected *Clostridium difficile* colonies. Through PNA probe melt curve analysis, we can make identification and toxigenic type characterization of *Clostridium difficile* isolates. We compared the performance of multiplex Real-time PCR with conventional PCR.

Results: Melting curve analysis showed that tpi, tcdA and tcdB have melt peak Tm 69 ± 2°C, 58 ± 2°C, and 69 ± 2°C, respectively. As a result of the analytical sensitivity test using the total DNA of *Clostridium difficile*, it was confirmed that the minimum detection limit could be detected up to 10 pg /ul. This result showed that the sensitivity was about 10 times higher than that of the conventional PCR.

Conclusions: Based on the results, the possibility of rapid and precise detection by a single experiment in a multiplex real-time PCR apparatus using the PNA probe for detecting and toxigenic typing of *Clostridium difficile* using feces has been shown.

P69

EVALUATION OF A NEW RANDOM-ACCESS ANTIGEN TEST FOR THE DETECTION OF *Clostridium difficile*

Savolainen Roosa[1], Koskinen Juha M.[2], Koskinen Janne O.[2], Kaukoranta Suvi-Sirkku[1]

[1]Department of Clinical Microbiology, Vaasa Central Hospital, Vaasa, Finland
[2]ArcDia International Ltd, Turku, Finland

The objective of this study was to evaluate the new automated random-access antigen detection test, mariPOC® CDI (ArcDia International Ltd, Finland), for the detection of *Clostridium difficile* GDH and toxins A and B directly from faecal specimens. mariPOC® was prospectively compared with the routinely used PCR method GenomEra® *Clostridium difficile* (Abacus Diagnostica Oy, Finland) and with the TECHLAB® C. DIFF QUIK CHEK COMPLETETM (Alere Inc., USA) membrane enzyme immunoassay (MEIA).

The study was performed during May to September 2017 in Vaasa Central Hospital in Finland. Leftover native faecal specimens, stored in faecal sample containers, routinely tested for *Clostridium difficile* toxin B gene by PCR were used. All specimens positive with either the mariPOC® CDI test or the toxin PCR, were further tested with the MEIA. In addition, 110 randomly selected negative specimens were also tested. True positive was defined as a specimen positive with at least two methods. Toxigenic culture was used to resolve discrepant results.

In total, 337 specimens were analyzed with the mariPOC® CDI test and PCR. Of these, 157 specimens were also tested with the MEIA. When the two antigen detection tests were compared with each other, the sensitivity of mariPOC® for GDH was slightly lower (95.2 %) compared to the MEIA (100.0 %) but no toxin positive cases were missed. The sensitivity of mariPOC® for toxins A/B was better (100.0 %) compared to the MEIA (87.1 %). When compared with the PCR, the sensitivity and specificity for the mariPOC® toxin A/B test was 83.8 % (31/37) and 100.0 % (298/298), respectively. In addition, there were two specimens positive with only PCR that should be confirmed with another PCR method. The negative predictive values were 98.0 % and 99.7 % for the mariPOC® toxin A/B and GDH tests, respectively, and 99.7 % for the PCR. The positive predictive value for mariPOC® toxin A/B was identical with the PCR (100.0 %).

The high throughput with high sensitivity and specificity make mariPOC® CDI test a useful new tool to detect toxigenic *Clostridium difficile* from faecal specimens. Similar negative predictive values with the PCR suggest that mariPOC® could also be used as a primary screening tool. The random-access analysis of samples and automated result interpretation are advantages compared to other antigen detection methods.

P70

DIAGNOSTIC ASSAYS IN SUPPORT OF PFIZER'S PHASE 3 *Clostridium difficile* VACCINE EFFICACY STUDY

Elfassy A[1], Jones CH[1*], French,R[2], Sharma P[1], Rajyaguru U[1], Johnson J[1], Bader M[1], Giordano Schmidt D[1], Lee P[1], Liang J[2], Nguyen H[2], Kalina W[1], Liberator P[1], Jansen KU[1], Anderson AS[1], Pride MW[1]*

[1]Pfizer Vaccine Research and Development and [2]Pfizer Biotechnology Clinical Development Statistics, Pearl River, NY, USA

Pfizer is currently conducting a Phase 3 study (Clover: *Clostridium difficile* Vaccine Efficacy tRial) to evaluate the efficacy of a vaccine composed of *Clostridium difficile* toxoids A and B in adults ≥50 years of age. The primary endpoint for this study is to demonstrate that the vaccine is effective in reducing the incidence of a primary episode of *Clostridium difficile* infection (CDI). To ensure accurate laboratory diagnosis of CDI, Pfizer is using a two-step algorithm for testing stool samples. This algorithm identifies toxigenic *Clostridium difficile* organisms by a nucleic acid amplification test, and toxins using Pfizer's novel cell cytotoxicity neutralization assay (CCNA). This approach (diagnosis by detection of both *Clostridium difficile* organisms and toxins in stool samples) is supported by studies conducted by Planche et al1 and Polage et al2 and in diagnostic guidance documents issued by the European Society of Clinical Microbiology and Infectious Diseases3 and the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America4.

In the algorithm's first step, diarrheal stool samples are tested for toxigenic *Clostridium difficile* via polymerase chain reaction test (PCR; Xpert® *Clostridium difficile*/Epi, Cepheid, Sunnyvale, CA). This highly specific and sensitive assay detects *Clostridium difficile* bacteria containing the toxin B gene. Prior to initiating clinical testing, we requalified this assay to assess its performance. In the second step, PCR+ samples are evaluated in Pfizer's novel automated and high-throughput CCNA for the presence of toxins. Highlights of the PCR qualification and CCNA validation and clinical validation studies for the PCR and CCNA, respectively, are described. Both assays met all prespecified acceptance criteria and are suitable for their intended use as diagnostics in *Clostridium difficile* vaccine efficacy and epidemiology studies.

** First two authors contributed equally*

P71

CLINICAL RELEVANCE OF CT VALUE IN *Clostridium difficile* INFECTION DIAGNOSIS

Andrea Aira [1], Csaba Feher [2], Izaskun Alejo [2], Elisa Rubio [2], Laura Muñoz [1], Alejandro Muñoz [2], Alicia Novoa[2], Climent Casals-Pascual [1], Josep Mensa [2], Alex Soriano[3]

[1]Barcelona Institute for Global Health (ISGlobal) - Hospital Clínic de Barcelona- Universitat de Barcelona, Barcelona, Spain [2]Hospital Clínic de Barcelona, Barcelona, Spain, [3]Hospital Clínic de Barcelona - Universitat de Barcelona - IDIBAPS, Barcelona, Spain.

Background and aims: *Clostridium difficile* infection (CDI) affects every year 123,997 patients in Europe and 7,600 patients in Spain. In our hospital the diagnosis of CDI is a two-step process, i.e., the detection of GDH followed by real time PCR (RT-PCR) to detect CD. The number of cycles required to reach the positivity threshold is known as the cycle threshold or Ct. This parameter has been related to the magnitude of bacterial load and to the amount of free C.difficile toxin in the colon. Nevertheless, the data on the relation between Ct and the expectable clinical evolution of CDI is inconclusive.

The aim of this study was to evaluate the clinical relevance of the Ct value in addition to the diagnostic confirmation of CDI.

Methods: In this retrospective observational study we present clinical and microbiological data obtained from 230 patients with confirmed CDI by RT-PCR in our hospital from September 2016 until February 2018. The variables analyzed were sex, age, clinical symptoms, time to symptoms resolution, recurrence of infection and death. The association of clinical variables, outcomes and the Ct value was established by Spearman's rank correlation coefficient (rho) in GraphPad Prism 5 software. The level of statistical significance (alpha) was 0.05.

Results: The median age of the population was 71 years (IQR: 58-81) and 44.3% were women. 55% of patients had fever and they presented a median of 4 depositions per day (IQR: 3-5). In 19.78% of cases patients had their leukocyte count was >15.000 cells/mm^3 and the median creatinine was 0.94 mg/dL (IQR: 0.6-1.64). The mean duration of symptoms was 6.7 days (SD: $\pm$7.16) and 16.09% had a recurrence. 30 days mortality was 10.87%, of which 5.2% were directly related to the infection. The median Ct value was 24 (IQR: 22-27) and it was indirectly correlated with the time to symptoms recovery (Spearman r=-0.21, P<0.01). However, Ct value was not associated with the mortality or recurrence rate.

Conclusions: Ct value from RT-PCR in CDI detection is inversely associated with the time to recovery of the CDI episode. Ct values can be useful for antibiotic treatment selection and also to establish its duration.

P72

TWO YEARS EXPERIENCE OF *Clostridium difficile* DIAGNOSIS

Sashka Mihaylova[1,2], *Kalinka Bozhkova*[1], *Georgi Slavov*[1], *Daniela Atanasova*[1]

[1]*Medical laboratory "LINA", Bulgaria;* [2]*University "Prof. Dr. Asen Zlatarov", Burgas, Bulgaria.*

Background and aims: *Clostridium difficile* is the most common cause of health care-associated diarrhoea in developed countries and is a major source of nosocomial morbidity and mortality worldwide. One notable change in the epidemiology of *Clostridium difficile* infections is the apparent increased incidence in communities that were historically considered to be at low risk.
The aim of this study is to describe the epidemiological characteristics of patients who requested *Clostridium difficile* testing and to discuss the significance of different positive combinations.

Methods: Stool samples of patients were tested for *Clostridium difficile* glutamate dehydrogenase (GDH), toxin A and toxin B. CerTest BIOTEC *Clostridium difficile* GDH+Toxin A+B one step combo card was used as a colour-detection chromatographic immunoassay. Patient records were reviewed retrospectively. The following data were collected: age, gender, test results, number of test repetitions, etc.

Results: Three hundred thirty-three patients were tested since January 2016. They were from 3 days to 78 years old. Gender distribution was almost equal. The majority of patients were ambulatory. Approximately three-fourths of all patients exhibited negative tests. The most prevalent combination among the positive groups was: GDH (+); toxin A (-); and toxin B (-); followed by GDH (+); toxin A (+); and toxin B (+).

Conclusions: *Clostridium difficile* has become a key pathogen among ambulatory patients. It is detected in all age groups. Medical specialists should request testing according to patient's ages. Interpretation of positive results depends strongly on patient history.

P73

THE IMPROVEMENT OF DIAGNOSTICS IN SUSPECTED *Clostridium difficile* INFECTION BY IMPLEMENTATION OF A GASTROINTESTINAL PATHOGEN MULTIANALYTE TEST

Krutova Marcela[1], Tkadlec Jan[1], Zajac Miroslav[1], Briksi Ales[1], Nyc Otakar[1] and Drevinek Pavel[1]

1. Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Czech Republic

Background and aims: In the Czech Republic, laboratory tests for microbiological investigation are selected by physicians. Due to the overlap of clinical symptoms in acute gastroenteritis, the selection of a right laboratory test to identify the cause of diarrhea may be difficult. We aimed to improve the laboratory diagnostics in patients with diarrhea and suspected *Clostridium difficile* infection (CDI) by implementation of a new multiplex antigen detection test. We also compared the test sensitivity for the detection of *Clostridium difficile* glutamate dehydrogenase (GDH) and toxins A/B with a commercial enzymatic immunoassay widely used in the Czech Republic.

Methods: Starting in April 2018, all diarrheal stool samples requested for CDI testing were tested by a fluorescence immunoassay (mariPOC® gastro and CDI test), which allows the detection of *Clostridium difficile* GDH and toxins A/B, Norovirus GII.4, Norovirus GI, Rotavirus, Adenovirus and Campylobacter spp. In parallel, a rapid membrane enzyme immunoassay (C. DIFF QUIK CHEK COMPLETE®), was used for the detection of GDH and toxins A/B. The presence of *Clostridium difficile* and toxins in all GDH positive samples was confirmed by toxigenic culture.

Results: From a total of 120 stool samples tested by the mariPOC® gastro and CDI tests, the following number of samples were positive: *Clostridium difficile* GDH (n=33); toxins A/B (n=18); Rotavirus (n=7); Campylobacter spp. (n=5) and Norovirus GII.4 (n=3). One sample revealed a double positivity for GDH and rotavirus. Using the C. DIFF QUIK CHEK COMPLETE®, 30 samples were positive for GDH and 15 were toxin positive. The sensitivity of the mariPOC® CDI test was higher compared to the C. DIFF QUIK CHEK COMPLETE® by 10.0% in the detection of GDH and by 20.0% in the detection of toxins A/B. Moreover, in 15 cases we detected other causative agents of diarrhea irrespective of the physician's test request, thus would left undetected if multiplex antigen test was not used.

Conclusions: The preliminary results showed that the implementation of a gastrointestinal pathogen multianalyte test has improved the microbiology diagnostics of patients with diarrhea. The detection of GDH at a higher sensitivity allows for the identification of *Clostridium difficile* excretors. The higher sensitivity for toxins A/B contributes to CDI diagnoses. In 15 cases we identified another cause of diarrhea although *Clostridium difficile* was the suspected pathogen.

P74

HOW IMPORTANT ARE MICROBIOLOGICAL DATA IN THE SURVEILLANCE OF *Clostridium difficile* INFECTIONS?

Krutova Marcela[1], Nycova Elka[2], Vojackova Sabina[3], Balejova Magda[4], Geigerova Lenka[5], Bartonikova Natasa[6], Tejkalova Renata[7], Havlinova Lenka[8], Vagnerova Iva[9], Paleckova Vera[10], Ryskova Lenka[11], Jezek Petr[12], Zamazalova Dana[13], Vesela Denisa[14], Kucharova Alice[15], Nemcova Dana[16], Curdova Martina[17], Drevinek Pavel[1] and Nyc Otakar[1].

1. Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Czech Republic; 2. Department of Medical Microbiology, Hospital Bulovka, Prague, Czech Republic; 3. Department of Medical Microbiology, University Hospital Brno, Czech Republic; 4. Department of Medical Microbiology, Hospital Ceske Budejovice, Czech Republic; 5. Department of Medical Microbiology, Faculty of Medicine and University Hospital Plzen, Charles University in Prague, Czech Republic; 6. Department of Medical Microbiology, Tomas Bata´s Hospital Zlin, Czech Republic; 7. Department of Medical Microbiology, Faculty of Medicine, Masaryk University and St. Anne's University Hospital Brno, Czech Republic; 8. Department of Medical Microbiology and Immunology, Hospital Liberec, Czech Republic; 9. Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University Hospital, Olomouc, Czech Republic; 10. Department of Medical Microbiology, Thomayer´s Hospital Prague, Czech Republic; 11. Department of Clinical Microbiology, University Hospital Hradec Kralove, Czech Republic; 12. Department of Clinical microbiology and parasitology, Hospital Pribram, Czech Republic; 13. Department of Clinical Microbiology, Hospital, Nove Mesto na Morave, Czech Republic; 14. Department of Medical Microbiology, Hospital Jindrichuv Hradec, Czech Republic; 15. Department of Medical Microbiology, Hospital Tabor, Czech Republic; 16. Department of Clinical Microbiology, Institute for Clinical and Experimental Medicine Prague, Czech Republic; 17. Department of Clinical Microbiology, Military University Hospital Prague, Czech Republic.

Background and aims: In 2015, the European Centre for Disease Prevention and Control (ECDC) released a protocol for a standardized surveillance of *Clostridium difficile* infections (CDI) in EU/EEA countries. An enhanced option of the surveillance includes microbiological data, but the number of *Clostridium difficile* isolates requested for further characterization decreased from ten (v 2.1) to five (v 2.3) CDIs for each hospital. We aimed to determine the benefit of characterizing the unrestricted number of *Clostridium difficile* isolates in the CDI surveillance.

Methods: Between October and December 2017, seventeen hospitals submitted epidemiological data according to the ECDC surveillance protocol v 2.3 and *Clostridium difficile* isolates on all CDI cases to Motol University Hospital, Prague. In *Clostridium difficile* isolates, a capillary-electrophoresis ribotyping was performed according to the new consensus protocol (Fawley et al., PONE, 2015). The antibiotic susceptibility of the isolates to metronidazole, vancomycin and moxifloxacin was determined by agar dilution method.

Results: The mean CDI incidence was 4.7 cases per 10,000 patient days. *Clostridium difficile* isolate and epidemiological data were available in 393 (84.2%) of 467 CDIs. Of 393 CDI cases, 295 (75.01%) were healthcare-associated (HA). The most prevalent PCR ribotypes (RTs) were 001 (n=127, 32.3%) and 176 (n=42, 10.7%). Seventeen isolates (RTs 001, 027, 176) and two isolates (RTs 001, 012) showed reduced susceptibility to metronidazole and vancomycin, respectively. A total of 46.3% (n=182) of isolates were resistant to moxifloxacin.

P74

Interestingly, 155 (52.5%) HA-CDIs were caused by RTs sporadically found during the study period. HA CDI, caused by less frequent RTs, also revealed a lower moxifloxacin resistance rate (11.0%), compared to HA CDIs caused by epidemic RTs 001, 027 and 176 (n=140, moxifloxacin resistance rate 91.6%).

Conclusions: Compared to previous Czech data, a decrease in the mean of CDI incidence and a lower prevalence of RT 176 *Clostridium difficile* was found. The detection of reduced susceptibility to metronidazole and vancomycin highlights the need for the screening of isolates of epidemic RTs for their susceptibility to first line CDI treatment drugs. The inclusion of microbiological data into the CDI surveillance allows us to get an objective view of CDI epidemiology in a healthcare environment.

P75

ROLE OF *Clostridium difficile* IN HOSPITAL ENVIRONMENT AND HEALTHCARE WORKERS

Váquez-Cuesta Silvia[1,3], Villar-Gómara Laura[1,2,3], Onori Raffaella[1,3], Alcalá Luis, Marín Mercedes, Muñoz Patricia[1,2,3,4], Bouza Emilio[2,3,4] and Reigadas Elena[1,2,3]

[1] Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain, [2] Medicine Department, School of Medicine, Universidad Complutense de Madrid (UCM), Madrid, Spain, [3] Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain, [4] CIBER de Enfermedades Respiratorias (CIBERES CB06/06/0058), Madrid, Spain

Background: *Clostridium difficile* infection (CDI) has traditionally considered to be transmitted within healthcare environment, from other patients or healthcare workers (HCW). Recently, this idea has been challenged. Our objective was to determine the extent of *Clostridium difficile* contamination and to establish potential transmission routes in a tertiary teaching hospital with a new methodology for *Clostridium difficile* recovery.

Methods: Environmental samples were taken (bed, WC, bathroom tap, door knob, alcohol gel device and call bell), from 3 different groups of patients: those with active CDI, colonized and negative for *Clostridium difficile* (control group). Environmental sampling was performed thrice per patient: at the time a fecal sample was taken for CDI diagnosis, 48 hours after, and 10 days after. HCW hands were also sampled. Samples were taken using a Polywipe ™ sponge device over the surface under study. The sponge side used for sampling was directly placed on the surface of a ChromID *Clostridium difficile* plate.

Results: During the study period (Jan 2018-ongoing), a total of 458 samples were taken, 13.3% were positive for toxigenic C.difficile (TCD) from a total of 31 patient wards (16 TCD negative patients [236 samples], 15 TCD positive patients [222 samples]). 17.6% of the wards of TCD positive patients were positive for TCD and 9.3% of the wards of TCD negative patients were positive for TCD (p=0.013). Out of the positive TCD patients, 11 had CDI and 4 were considered colonized. We observed no significant differences in environmental contamination between rooms from colonized and symptomatic patients(12.1% vs 19.9%; p=0.183). When cases were analyzed by sampling time, at diagnosis 50% were positive, 27.3% were positive at 48h after symptom resolution (when contact precautions/isolation measures were removed) and 27.3% were positive after course of treatment. Overall, the most contaminated site corresponded to the WC, followed by the call bell. 4.2% of HCW hands were colonized.

Conclusions: We found a significant proportion of surfaces contaminated with TCD, as well as hand colonization of HCW. It is notable that when isolation/precaution measures ended, in those wards remained a significant proportion of contaminated sites. We were not able to document any epidemiological transmission link during the study period.

P76

Clostridium difficile PREVENTION AND MANAGEMENT : AN ASSESSMENT OF CURRENT CLINICAL PRACTICE PATTERNS OF PHYSICIANS

Lubarda Jovana[1], Maeglin John[1], Mathews Debra[2], Delk Trudi[2], Septimus Edward[3]

[1]Medscape LLC, New York, United States; [2]Synthetic Biologics, Maryland, United States; [3]Texas A&M College of Medicine, Internal Medicine, Houston, United States

Background and aims: This aim of this study was to investigate physicians' current practice patterns, knowledge, and competence in CDI prevention and management.

Methods: A clinical practice assessment consisting of 25 multiple-choice knowledge- and case-based questions was made available to physicians in multiple specialties, including infectious disease, emergency medicine, surgery and gastroenterology, in the United States who encounter patients with CDI. There was no monetary compensation or charge for participation. Questions evaluated knowledge, competence, skills, barriers, and attitudes related to CDI, such as recognition of risk factors, strategies for limiting risk, and emerging strategies for prevention. The assessment launched online on a website dedicated to continuous professional development on October 27, 2017. Data were collected until January 16, 2018. Respondent confidentiality was maintained and responses were de-identified and aggregated prior to analyses

Results: 1115 physicians completed the survey during the study period. Key findings include: 69% were not aware of the incidence of CDI in the United States. 72% reported at least one case of CDI occurring in their practice over the past year. While 70% correctly identified antibiotics most closely associated with development of CDI, only 8% reported they were very confident in recognizing host risk factors for CDI. Moreover, 75% were not aware of the risks of CDI-associated death in older patients versus middle-aged patients. 43% use a polymerase chain reaction–based method for CDI diagnosis; 29% use a 2-step method combining different test types. 57% were not aware of the relationship between the gut microbiome and CDI. About 53% were not aware of new strategies being investigated for prevention of CDI and their mechanisms of action. The most important goals of antimicrobial stewardship as reported by the physicians were: achieving optimal clinical outcomes reported by 53%; limiting selection for antimicrobial-resistant strains reported by 25%; and, minimizing toxicity and other adverse events reported by 18%

Conclusions: This research yielded important insights into current clinical practices of physicians and gaps in the prevention and management of CDI which could inform development of educational initiatives for future medical education projects.

P77

MOLECULAR EPIDEMIOLOGY OF *Clostridium difficile* ISOLATES FROM A UNIVERSITY HOSPITAL OF BRAZIL

Rodrigo O. S. Silva[1], Amanda N. Diniz[1], Carlos A. Oliveira Júnior[1], Eduardo G. Vilela[2], Henrique C. P. Figueiredo[1], Mark H. Wilcox[3], Warren N. Fawley[3], Dominique Stéphane Blanc[4], Francisco Carlos Faria Lobato[1]

[1]Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil; [2]Faculdade de Medicina, Universidade Federal de Minas Gerais, Brazil; [3]University of Leeds, United Kingdom; [4]Hospital Preventive Medicine, Lausanne University hospital, Lausanne, Switzerland

Since the early 2000s, the incidence and severity of *Clostridium difficile* infection (CDI) have increased dramatically in some countries. This finding has been linked to the emergence of so-called hypervirulent strains, such as those from PCR ribotype (RT) 027 and RT078, which are classified by multilocus sequence typing (MLST) in clades 2 and 5. So far, studies in several different Brazilian hospitals failed to detect ribotypes 027 and 078. On the other hand, some works have identified several cdtB-positive strains in humans and animals in Brazil, some from new ribotypes. These reports raised the hypothesis that some novel hypervirulent strains might be circulating in Brazilian hospitals. Thus, the aim of this study was to evaluate 34 non-duplicate *Clostridium difficile* isolates by MLST in order to better understand the epidemiology of *Clostridium difficile* infection (CDI) in a Brazilian hospital. Toxigenic *Clostridium difficile* isolates were obtained from inpatients with confirmed CDI at the Clinical Hospital of the Federal University of Minas Gerais from 2012 to 2017. All isolates were subjected to MLST and all new strain types (ST) were PCR ribotyped. MLST revealed 19 STs, including six novel STs and five new allele sequences. Of these six novel STs, four were identified also as new ribotypes. This diversity seems higher than previously reported in other hospitals elsewhere. Noteworthy, a high percentage of new STs were identified among the tested strains (31.6%), which also suggests an unusual epidemiology of CDI in our institution. ST42 (all RT106), and ST2 (all RT014/020) were the most common strain types in the present study, being detected in eight (23.5%) and four (11.8%) strains, respectively. Six strains (17.6%) were classified in clade 2. Except for one strain, identified as ST114/RT111, all other isolates from clade 2 in the present study were classified into novel STs and confirmed as new ribotypes. While most previously studies on Brazil focused on the absence of RT027 or RT078 in humans, our study suggests that other possible hypervirulent strains might be circulating in Brazilian hospitals. Thus, the present work highlights the importance of permanent vigilance and reinforce the need for broader epidemiological studies in Brazil to characterize currently circulating *Clostridium difficile* strains at a national scale.

P78

Clostridium difficile INFECTION IN SWEDEN: RESULTS FROM THE NATIONAL SURVEILLANCE PROGRAM 2009-2016.

Kristina Rizzardi[1], Torbjörn Norén[2], Olov Aspevall[1], Barbro Mäkitalo[1], Michael Toepfer[3], Åsa Johansson[4], Thomas Åkerlund[1]

[1]Public Health Agency of Sweden, Solna, Sweden; [2]Örebro University, Örebro, Sweden ; [3]Unilabs Clinical Microbiology, Skövde, Sweden; [4]Växjö Hospital, Sweden

Background and aim: A Swedish national surveillance program for CDI was initiated in 2009, aiming to monitor the apparent nationwide increase in CDI cases, detect trends and outbreaks, and determine the baseline incidence of CDI in the catchment area of the local clinical laboratories. The aim of this study is to summarize the Swedish national surveillance program with regard to incidence rates, distribution of *Clostridium difficile* types including known outbreaks and the impact of diagnostic methods on CDI incidence.

Method: Epidemiological case data was collected through a voluntary reporting system. Epidemiological typing using PCR ribotyping and antimicrobial susceptibility testing was performed twice a year on isolates sent from the local clinical laboratories. The information regarding the current diagnostic method used by local laboratories was also collected with the isolates.

Result: The national CDI incidence decreased by 22% between 2012 and 2016 and the proportion of multi-drug resistant (MDR) isolates decreased by 80%. Furthermore, the variation in incidence between counties also diminished over the period, In contrast to other studies we did not observe an increased incidence due to the introduction of NAAT.

Conclusion: Our results suggest that successful implementation of hygiene measures is the major cause of the observed incidence decrease. While decreased antibiotic consumption or prudent use may be part of the explanation we suggest that the major impact is due to improved hygiene measures in health care and hospital settings. This hypothesis is supported by (i) sales of typical risk antibiotics in hospitals, where CDI is predominant, were virtually unchanged during the time period, (ii) a substantial reduction in CDI cases occurred among elderly patients known to be hospitalized to a larger extent, (iii) the apparent disappearance of geographical clusters of specific *Clostridium difficile* PCR ribotypes, indicative of reduced nosocomial spread. However, since cases are not classified into community- and health care associated CDI, we cannot entirely rule out the possibility that the observed incidence reduction occurred mainly in the community, where antibiotic sales have decreased significantly more compared to in-patient sales.

P79

MOLECULAR EPIDEMIOLOGY AND ANTIMICROBIAL RESISTANCE PROFILES OF *Clostridium difficile* STRAINS IN THE UNITED STATES BETWEEN 2011-2017

Tickler, Isabella A.[1], Obradovich, Anne E.[2], Goering, Richard V.[2], Tenover, Fred C.[1], the HAI Consortium

[1]Cepheid, Sunnyvale, CA, United States, [2]Creighton University School of Medicine Omaha, NE, United States

Background: The molecular epidemiology of *Clostridium difficile* continues to evolve in the US with new strain types replacing epidemic PCR ribotype 027. In this study, we compared the PCR ribotypes and antimicrobial susceptibility patterns of *Clostridium difficile* isolates collected from US hospitals between 2011 and 2017.

Methods: 943 toxigenic *Clostridium difficile* were isolated from stool samples received from 26 laboratories in the US during three collection times: 2011-2012, 2013-2014 and 2015-2017. Isolates underwent PCR ribotyping and were tested for resistance to moxifloxacin (MX), metronidazole (MT), clindamycin (CC), rifampin (RI), tetracycline (TC) and vancomycin (VA) using the Etest method. Fisher's exact test was used to compare the distribution of ribotypes and changes in antibiograms over the three periods.

Results: Overall, 64 ribotypes (RT) were identified among the isolates. RT027 was the predominant strain type in 2011-2012 and 2013-2014 (30% and 23%, respectively) followed by 014/020, 106, and 002. However, in 2015-2017, RT106 accounted for 20% of the ribotypes detected, followed by 014/020, 027, and 056. The proportions of all other ribotypes remained steady, with the exception of RT056, which increased in prevalence from 3% (2011-2012 and 2013-2014) to 10% in 2015-2017 ($p < 0.0001$ and $p = 0.002$, respectively).

All isolates were susceptible to MT, while 11% of isolates tested showed reduced susceptibility to VA (MIC > 2 µg/mL but ≤6 µg/mL). Among RT027 isolates, MX resistance decreased from 93% and 95% (2011-2012 and 2013-2014, respectively) to 88% in 2015-2017 ($p = 0.296$ and $p = 0.236$, respectively), while TC resistance increased from 6% and 5% to 21% ($p = 0.014$ and $p = 0.030$, respectively). Multi-drug resistance (resistance to CC, MX, RI and TC) was observed in 21% of RT027 isolated in 2015-2017, a significant increase over the 3% and 5% proportions observed in prior surveys ($p = 0.002$ and $p = 0.030$).

Conclusions: The distribution of PCR ribotypes among *Clostridium difficile* isolates in the US continues to evolve. In the six years of our survey, we observed a decrease in prevalence of RT027 isolates accompanied by an increase of RT106. In a prior study, we reported a rise in prevalence of RT014/020, but in the current survey RT014/020 frequency has remained steady. Although RT027 appears to be declining, multi-drug resistance in RT027 and RT017 continues to be common.

P80

THE BURDEN OF *Clostridioides difficile* INFECTION IN JAPAN: A PROSPECTIVE MULTI-CENTER STUDY

Kato Haru[1], Senoh Mitsutoshi[1], Honda Hitoshi[2], Tagashira Yasuaki[2], Dubberke Erik R.[3], Reske Kimberly[3], Olsen Margaret A.[3] and The Clostridium difficile infection Japan Study Group

National Institute of Infectious Diseases, Tokyo, Japan; Tokyo Metropolitan Tama General Medical Center, Tokyo, Japan; Washington University School of Medicine, St. Louis, Missouri, USA

Background: Retrospective studies in Japan have found lower incidence of Clostridioides difficile infection (CDI) than reported from North America. There are several potential explanations, including strain distribution and testing practices.

Methods: A prospective cohort study of CDI was conducted from May 2014 – May 2015 at 12 medical facilities (total 20 wards) in 11 prefectures in Japan. Patients who had at least 3 diarrheal bowel movements (Bristol stool grade 6-7) in the prior 24 hours were enrolled. CDI was defined as positive for any of tests: enzyme immunoassay for toxins A/B, nucleic acid amplification test detecting the toxin B gene, or toxigenic culture. Recovered *Clostridium difficile* isolates underwent PCR-ribotyping (RT), slpA-sequence typing (slpA-ST), and antimicrobial susceptibility testing.

Results: Among the 636 enrollment diarrheal episodes, 173 met the definition of CDI. The overall incidence rate was 7.35/10,000 patient-days (pd). The incidence was high in 5 ICU wards (rate=22.21 CDI cases per 10,000 pd range, 13.91-75.53/10,000 pd). There was a correlation between testing frequency and CDI incidence rate (R^2=0.91). Of the 173 CDI episodes, 146 were positive by toxigenic culture. RT 018/018" was most dominant (29%), followed by types 014 (23%), 002 (12%), and 369 (11%). These 4 types corresponded to 75% of the recovered isolates. Among the 15 non-ICU wards, two had high CDI incidence rates (13.03 and 15.92 CDI cases per 10,000 pd), with clusters due to RT 018/slpA-ST smz-02 and 018" /smz-01, respectively. All isolates belonging to RT 018/018" were resistant to moxifloxacin, gatifloxacin, and clindamycin but susceptible to vancomycin, metronidazole and rifampicin. Three non-RT027 or 078 binary toxin-positive isolates were isolated.

Conclusions: In this study laboratory testing rates were correlated with detection of CDI in hospitalized patients with diarrhea, suggesting that a sizable number of patients with CDI are not identified due to low testing rates in Japan. It is suggested that selective pressure by overuse of antimicrobial agents leads to the spread of RT 018/018". It is important to raise the awareness of clinicians and infection control for CDI in Japan, including optimal laboratory diagnosis and implementation of antimicrobial stewardship programs.

P81

PREVALENCE OF *Clostridium difficile* RIBOTYPES IN NORTHERN IRELAND 2009 – 2018

MC Connor[2], JP McKenna[1], L. Crawford[1], L. Neill[1], G. McMullan[2], DJ Fairley[1]

[1]Department of Microbiology, Belfast Health & Social Care Trust, Belfast, Northern Ireland; [2]School of Biological Sciences and the Institute for Global Food Security, Medical Biology Centre, Queens University Belfast, Northern Ireland

Introduction: Belfast Health & Social Care Trust (BHSCT) currently provides regional *Clostridium difficile* (CD) culture and ribotyping services for Northern Ireland (NI). In contrast to the other UK members of the *Clostridium difficile* ribotyping network (CDRN), all suspected CD toxin positive stool samples in NI undergo culture with all identified CD isolates subsequently ribotyped. This high level of surveillance is unique with ribotype data available for every confirmed CDI case in NI (population 1.8 million) over a nine-year period. Materials and methods: Suspected CD toxin positive stool specimens were received by Department of Microbiology BHSCT from 2009 – 2018 from healthcare facilities across NI region. Stool specimens underwent toxigenic culture with all confirmed CD isolates characterized using capillary gel electrophoresis based ribotyping.

Results: A total of 8825 CD cases were documented in NI from 2009-2018. The data has shown that the most prevalent ribotypes causing human infection appear to be relatively stable with ribotypes 078, 001, 014, 002, 020, 015, 005 and 193 consistently falling within the top ten most common ribotypes. Ribotype 078 was consistently the number one ribotype isolated from humans in NI, with the exception of 2017, where ribotype 002 was identified as the most prevalent. The epidemic ribotype 027 was seen across the data set in consistently low prevalence ranging from 2.4% in 2009 to 0.5% in 2017.

Discussion: This study has shown the ribotype distribution in NI appears to be relatively stable with a number of 'key stone' ribotypes consistently in circulation. Other ribotypes were observed to come in and out of circulation, which possibly indicates that external sources contribute to ribotype diversity. The continued surveillance of C. difficile, and particularly potentially epidemic strains such as ribotype 078, is imperative to in understanding this pathogen's distribution with the aim of potentially curtailing transmission.

P82

Clostridium difficile INFECTION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: A CASE CONTROL STUDY

Krista Vitikainen[1], Johanna Haapamäki[1], Martti Färkkilä[1], ,Perttu Arkkila[1] and Veli-Jukka Anttila[2]

[1]Department of Gastroenterology, Helsinki University Central Hospital, Helsinki, Finland
[2]Department of Infectious Diseases, Helsinki University Central Hospital, Helsinki, Finland

Inflammatory bowel disease (IBD) patients are at increased risk for developing symptomatic *Clostridium difficile* infection (CDI). In previous studies IBD patients have been more susceptible to have recurrences of CDI (rCDI). However, predisposing factors for rCDI in IBD patients are poorly established. We studied the occurrence of recurrences and predisposing factors for rCDI in IBD and compared the differences of CDI in patients with and without IBD. A retrospective IBD-CDI cohort (n=167) between 2008-2013 from Helsinki University Hospital register was gathered. Patient characteristics were compared with age- and gender-matched control group including non-IBD CDI-patients. Clinical parameters, including mortality, CDI episodes and O27 ribotype were registered. We found no difference in rCDI between IBD-CDI and control CDI patient cohorts. As compared with IBD subtypes, rCDI was least common among patients with Crohn's disease. The use of systemic corticosteroid seemed to increase the risk for rCDI in IBD. Proportionally, 5-ASA consumption also increased among IBD-CDI patients with two or more CDI episodes. The prevalence of 027-ribotype and mortality rates did not differ significantly among the cohorts. None of the IBD patients underwent colectomy upon CDI. Higher 5-ASA and corticosteroid-intake was associated with higher risk of rCDI in IBD patients. No significant difference was identified in the rCDI rates between IBD-CDI and CDI cohorts. The prevalence of 027-ribotype of *Clostridium difficile* was slightly higher in non-IBD CDI cases, although statistical significance was not reached. In our cohort of IBD, CDI was not significantly associated with poor prognosis since mortality rates did not differ among the groups and none of the patients in IBD-CDI cohort underwent colectomy after CDI.

P83

INTESTINAL COLONIZATION OF *Clostridioides difficile* IN PEDIATRIC INFLAMMATORY BOWEL DISEASE PATIENTS IN JAPAN

Murabata Mayumi [1,2], Kato Haru [3], Senoh Mitsutoshi[3], Uchida Keiichi[4], Inoue Mikihiro[4], Koike Yuhki[4], Matushita Kohei[4], Nagano Yuka[4], Yano Hisako[5]

[1]Mie University, Course of Nursing Science, Graduate School of Medicine, Mie, Japan; [2]Mie National Hospital, Institute for Clinical Research, Mie, Japan; [3]National Institute of Infectious Diseases, Tokyo, Japan; [4]Mie University Hospital, Department of Pediatric Surgery, Mie, Japan; [5]Nagoya City University, Graduate School of Nursing, Aichi, Japan

Background. Clostridioides difficile infection (CDI) has been reported to complicate the course of inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD). Pediatric IBD patients have more aggressive and extensive diseases than the adults, and consequently they require more antibiotics, corticosteroids and immunosuppressive drugs. The management of CDI may be more crucial in children with IBD, however, there are few studies about the role of *Clostridium difficile* in pediatric IBD in Japan. In this study, we investigated intestinal colonization of *Clostridium difficile* in pediatric IBD patients, and its correlation with disease activity of IBD.

Methods. Eleven UC patients and one CD patient (5-15 years of age, average age: 10.7 years) were examined. *Clostridium difficile* was cultured from their stool specimens and toxin-producing type of recovered isolates was tested by PCR detecting the genes encoding toxin A, toxin B and binary toxin. The isolates were analyzed by PCR ribotyping. Pediatric Ulcerative Colitis Activity Index and Pediatric Crohn 's Disease Activity Index were used to categorize the IBD condition in patients with UC and CD, respectively, when stool specimens were collected.

Results. A total of 48 stool specimens were obtained from the 12 patients. Disease activity index ranged 0 to 85 points (average 17.5 points) among 11 UC patients, and was 15 points in one CD patient. During the study period, none of the 12 patients were given the diagnosis of CDI. Of 46 specimens from UC patients, 17 were positive for *Clostridium difficile*. Among 17 isolates recovered from 3 UC children, 6 different PCR-ribotypes were identified. The child with CD was examined twice, and *Clostridium difficile* was recovered on one of the occasions. Of 18 isolates obtained, only one from a UC patient was toxin A-positive, toxin B-positive binary toxin-negative and typed as PCR-ribotype 014, and the remaining 17 were all non-toxigenic. The disease activity scores in 4 patients when *Clostridium difficile* was isolated were low from 0 to 15 points.

Conclusions. In the 12 subjects examined here, no correlation between *Clostridium difficile* colonization and disease activity of IBD was observed. Further studies are required to clarify the impact of CDI and *Clostridium difficile* colonization on disease severity of IBD in children.

P84

EPIDEMIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF *Clostridium difficile* IN PIGLETS IN THAILAND

Putsathit Papanin[1], Ngamwongsatit Bhinyada[2], Riley Thomas[3,4,5]

[1]The University of Western Australia, School of Biomedical Sciences, Nedlands, Western Australia, Australia; [2]Mahidol University, Faculty of Veterinary Science, Nakhon Pathom, Thailand; [3]PathWest Laboratory Medicine, Nedlands, Western Australia, Australia; [4]Edith Cowan University, School of Medical and Health Sciences, Joondalup, Western Australia, Australia; [5]Murdoch University, School of Veterinary and Life Sciences, Murdoch, Western Australia, Australia.

Background and aims: *Clostridium difficile* is an important enteric pathogen of neonatal pigs. To date, the epidemiology of *Clostridium difficile* infection (CDI) in piglets in Thailand is unknown. This study aimed to investigate the prevalence, molecular epidemiology and antimicrobial susceptibility of *Clostridium difficile* in piglets and the piggery environment in Thailand, and to describe characteristics associated with *Clostridium difficile* positivity in piglets.

Methods: Piglet rectal swabs (n=165) and piggery environmental specimens (n=9) were collected in 2015 from five farms located in the central region of Thailand. All specimens were tested for the presence of *Clostridium difficile* with toxigenic culture. PCR assays were performed on isolates to investigate ribotype (RT) and the presence of toxins A and B, and binary toxin genes. The susceptibility of *Clostridium difficile* to nine antimicrobials was tested using an agar incorporation technique. Laboratory results were analysed in combination with epidemiological data.

Results: *Clostridium difficile* was isolated from 35.2% (58/165) of the piglets and 88.9% (8/9) of the environmental specimens. All strains were non-toxigenic. The most common strain belonged to a novel RT QX083, which accounted for 87.9% (51/58) and 77.8% (7/9) of the isolates from piglets and the environment. The hyper-virulent RT 078 strain was not found. Resistance against fidaxomicin, vancomycin, metronidazole, rifaximin, amoxicillin/clavulanate and meropenem was not observed. High levels of resistance against clindamycin (98.3% and 100%, respectively), erythromycin (75.9% and 66.7%, respectively) and moxifloxacin (51.7% and 55.6%, respectively) were seen among isolates from piglets and the environment. *Clostridium difficile* positivity in piglets was age dependant (OR 0.88, p=0.001), and the prevalence was 45.2%, 39.6% and 0% among piglets aged 1-7, 8-14 and 15-23 days old, respectively. C. difficile was more common among non-diarrhoeic than diarrhoeic piglets (64.9%; 37/57 vs. 35.1%; 20/57), but not significantly so (p=0.077, $\chi 2$ test). Piglets born to older or higher parity sows were more likely to be colonised (OR 1.05, 95% CI 1.01-1.09, p=0.007 and OR 1.16, 95% CI 1.02-1.33, p=0.030, respectively).

Conclusions: The high prevalence of antimicrobial resistant strains suggests that *Clostridium difficile* could act as a reservoir of resistance genes in the community.

P85

A NATIONWIDE STUDY OF MOLECULAR EPIDEMIOLOGY AND ANTIBICROBIAL RESISTANCE OF *Clostridium difficile* IN SOUTH KOREA

Heejung Kim[1], Seok Hoon Jeong[1], Jeong Hwan Shin[2], Young Ah Kim[3], Jong Hee Shin[4], Kyeong Seob Shin[5], Uh Young[6]

[1]Yonei University College of Medicine, Seoul, South Korea; [2]Inje Universtiy Busan Paik Hospital, Busan, South Korea; [3]National Health Insurance Service Ilsan Hospital, Goyang, South Korea; [4]Chonnam National University Medical School, Gwangju, South Korea; [5]Chungbuk National University College of Medicine, Cheongju, South Korea; [6]Yonsei University Wonju College of Medicine, Wonju, South Korea

Background and aims: Recently, awareness of *Clostridium difficile* infection in Korea has been increasing widely. To assess the molecular epidemiology and antimicrobial resistance of *Clostridium difficile* in South Korea as part of a National Surveillance System.

Methods: From Feb to May 2017, non-duplicated isolates of *Clostridium difficile* collected from referral hospitals representing the 6 regions in South Korea. We performed toxin gene PCR, PCR ribotyping, multilocus sequence typing (MLST), antimicrobial susceptibility by agar dilution test according to the recommendations of the CLSI, detection of antimicrobial resistance gene such as chloramphenicol resistance gene catD, erythromycin resistance gene ermB, tetracycline resistance protein tetM, glycopeptide resistance vanZ and nitroimidazole reductase nimR by PCR.

Results: Of the total 331 *Clostridium difficile* isolates, 257 (77.6%) were toxigenic. The prevalence of the strain producing binary toxin (CDT) was 3.9% (13/331). A total of 53 different ribotype patterns were found; A+B+ CDT- 28 types, A-B+CDT- 1 type, A+B+CDT+ 7 types, A-B-CDT- 17 types. Among them, 11 types were newly classified. Ribotype 018 was the most common ribotype (25.1%), and Ribotype 014/020, Ribotype 002 and Ribotype 012 were common. MLST analysis of *Clostridium difficile* identified 39 sequence types, of which 3 types of STs were new not in the pubmed library. R018 ST17, R002 ST8, R012 ST54, and R017 ST37 were common. Resistance rates for ampicillin, cefotetan, clindamycin, imipenem, chloramphenicol, tetracyclin, moxifloxacin and rifaximin were 50%, 39%, 61%, 49%, 0%, 5%, 44% and 21%, respectively. All strains were susceptible to metronidazole and vancomycin for the treatment of *Clostridium difficile* infection. PCR ribotype 018, 002, and 017 showed high MIC to various antimicrobial agents and strains with multi-drug resistance were also common. The positive rates of catD, ermB, tetM, vanZ and nimR gene were 0%, 74.2%, 11.7%, 82.5% and 100%, respectively.

Conclusions: R018 ST17 was the most prevalent at 5 hospitals and showed high resistance rate to ampicillin, cefotetan, clindamycin, imipenem, and moxifloxacin. All A^-B^+ strains showed R017 ST37, accounted for 4.8% of all isolates. The prevalence of binary toxin-producing strains was 3.9%. We did not isolate strains with decreased susceptibility to metronidazole or vancomycin.

P86

INVESTIGATING RECURRENT *Clostridium difficile* INFECTION IN WESTERN AUSTRALIA USING LINKED DATA

Collins Deirdre A[1], Marcella Stephen[2], Riley Thomas V[1, 3, 4]

[1] School of Medical & Health Sciences, Edith Cowan University, Joondalup, Australia
[2] Merck & Co., Inc., Kenilworth, NJ, USA
[3] School of Veterinary & Life Sciences, Murdoch University, Murdoch, Australia
[4] Department of Microbiology, PathWest Laboratory Medicine, Nedlands, Australia

Background and aims: Since January 2010, mandatory reporting of *Clostridium difficile* infection (CDI) has occurred in all public hospitals in Western Australia (WA). These data can be linked with many medical administrative datasets available for research purposes using a unique medical record number. The aim of this study was to describe characteristics of CDI patients in WA, with a particular focus on patients experiencing repeated episodes of CDI.

Methods: CDI cases were defined as diarrhoea (loose or watery stool) with a positive diagnostic PCR for tcdB. All CDI cases recorded from July 2012-June 2014 in the Healthcare Infection Surveillance WA dataset for three hospitals in the Perth North Metropolitan Health Service were included in the study. CDI case records and molecular typing data were linked with their corresponding hospital morbidity records on the Patient Administration System. Where available, isolates for each CDI episode were PCR ribotyped. Incidence rates were calculated, considering CDI episodes recorded ≥8 weeks apart for the same patient as new cases. Recurrent CDI was defined as ≥2 CDI episodes recorded within a 1-8 week period. These were further defined as relapse when corresponding *Clostridium difficile* isolates were of the same ribotype (RT), and reinfection where isolates were different RTs.

Results: There were 367 individual cases of CDI identified among 332 patients who experienced ≥1 CDI episode during the study period. The overall incidence rate was 4.64/10,000 patient days. The median age of cases was 73.6y, 57.8% were female. 353 unique (excluding recurrent cases) *Clostridium difficile* isolates were recovered representing 77 different RTs. RT 014/020 predominated (37.1%), followed by RTs 056 (7.1%), 002 (6.2%) and 018 (2.8%). In total, 41 patients (12.4%) had ≥1 episode of recurrent CDI; 72.2% were relapse episodes and 36.0% reinfections. 31 patients had ≥2 episodes of CDI recorded ≥8 weeks apart; 25 had multiple isolates (2-5) collected. Notably, 80% of these patients had the same RT recorded for each episode at intervals ranging from 66-711 days.

Conclusions: The incidence of CDI in WA is high and RT 014/020 continues to be the dominant molecular type in an otherwise diverse array of strains. The high strain diversity and high proportion of reinfections among recurrent CDI cases suggests CDI cases arise from exposure to many different reservoirs.

P87

PCR-RIBOTYPE VARIABILITY OF *Clostridium difficile* STRAINS FROM THE PATIENTS WITH HOSPITAL-ACQUIRED *Clostridium difficile* INFECTIONS (HACDI), COMMUNITY-ACQUIRED CDI (CACDI), TOXIGENIC COLONIZATION AND NON-TOXIGENIC COLONIZATION.

Mi-Ran Seo, Jieun Kim, Yang-soon Lee[1], Bongyeong Kim, Hyunjoo Pai

Department of Internal Medicine and Laboratory Medicine[1], Hanyang University College of Medicine, Seoul, Korea

Background and Aim: According to the pathophysiology of CDI, the strains from the patients with HACDI and CD colonization would be similar in an institution. However, few data presented direct comparison of ribotype distribution of the CD strains from CDI and colonization in an institution. In order to help understanding the epidemiology of CDI in a hospital, we compared the PCR ribotypes of CD strains from HACDI and toxigenic colonization as well as from CACDI and non-toxigeneic colonization using the stool samples submitted for CD cultures in an institution during 3 years.

Methods: All CD strains from stool submitted for CD cultures in Hanyang University Hospital during the year of 2009, 2012 and 2014 were included. Detection of toxin genes using multiplex PCR and PCR ribotyping were performed as described previously, and restrospective chart review was performed as well. According to the carriage of toxin genes and results of chart review, the enrolled patients were categorized into the groups of CACDI, HACDI, toxigenic colonization and non-toxigenic colonization.

Results: During the 3 years, 757 CD strains were identified from 757 patients. Among the 757 patients, 20 were CACDI, 462 HACDI, 141 toxigenic carriers and 134 non-toxigenic carriers. Common RTs were RT017 (25%), RT112 (15%), RT012 (10%), RT015 (10%), RT018 (5%), unkown ribotype 04 (UNK04) (5%), UNK07 (5%) and UNK13 (5%) in CACDI; RT018 (35%), RT017 (13%), RT002 (8%), RT001 (6.5%), RT015 (5.6%), RT014 (3.7%), RT112 (2.4%), and more in HACDI; RT018 (26.2%), RT017 (12.8%), RT012 (10.6%), RT112 (7.8%), UNK03 (5.7%), UNK01 (3.5%), UNK14(3.5%), RT001(2.8%) and more in toxigenic colonization; all unkown ribotypes except one RT018 in non-toxigenic colonization. One strain (5%) from CACDI and 9 (1.8%) from HACDI were not identified from colonized patients, and 12 (8.4%) from toxigenic colonization did not make infections. Although most strains from non-toxigenic colonization were unknown ribotypes, they were also clustered according to the PCR ribotypes (maximum 14 strains showed the same PCR ribotypes).

Conclusions: Although RT017 and RT018 were most common both in HACDI and toxigenic colonization, other PCR ribotypes of CD strains from CACDI, HACDI, toxigenic colonization and non-toxigenic colonization varied in an institution during the same year.

P88

Clostridium difficile (CD) CARRIERS: RISK FACTORS AND INCIDENCE OF CD INFECTIONS (CDI)

Eyal Melzer[1], Gil Smollen[2], Amit Hupert[3], Ronen Fluss[3], Ilana Tal[4], Maayan Gilboa[5], Olga Field[6], Naty Keller[2], Galia Rahav[7] and Gili Regev-Yochay[4] for the SHIC research group

[1]Internal Med C, Sheba Medical Center, [2]Microbiology lab, Sheba Medical Center, [3]Biostatistical Unit, Gertner Research Institute, [4]Infection Prevention & Control Unit, Sheba Medical Center, [5]Internal Med D, Sheba Medical Center, [6]Internal Med F, Sheba Medical Center, [7]Infectious Dis Unit, Sheba Medical Center

Background: The role of asymptomatic carriers in CDI epidemiology is not fully understood. Here, we define the carriage prevalence on admission, factors associated with carriage, and the incidence among carriers and non-carriers.

Methods: During 10 weeks, all patients admitted on weekdays, to any of the seven Internal Medicine wards were screened for CD carriage, by PCR using Cepheid, Xpert® CD. Patients who developed diarrhea after at least 72h from admission were defined as hospital-onset CDI (HO-CDI), or hospital-acquired-CDI (HA-CDI), depending on carriage status on admission. Data on previous hospitalization, comorbidities and disability status were collected from the medical files.

Results: Of 3550 applicable admission, 2605 (73%) agreed to be screened, and results were available for 2360 swabs. Of these, visible fecal matter (VFM), i.e. „brown" swabs was present in 780 (30.9%) whereas 1580 (69.1%) appeared to be „white", i.e. without VFM. Older age and previous hospitalizations were associated with being a carrier. CD was detected in 85 individuals (3.6% of all available swabs). Yet, among patients with a "brown" swab, carriage was significantly higher (5.4% vs. 3.0% respectively, P=0.04). In total, 14 patients were diagnosed as clinical CDI during the study period. Carriage status on admission was available for 11/14; 4 CDI cases developed among carriers and 7 among non-carriers, yet only 2 of these non-carriers had a VFM-positive swab. Thus, the incidence of CDI was 47/1000 admissions among carriers and 0.8-3/1000 among non-carriers depending on the validity of the VFM-negative swabs (RR 15.3-58.6 for carriers to develop CDI).

Conclusions: Carriage of CD was detected in 3.8% of admitted patients, but carriage may be as high as 5.4%, if assessing only samples with VFM. Carriage was associated with older age and previous hospitalization and carriers had significantly increased risk to develop CDI.

P89

A TIME SERIES ANALYSIS OF RESPIRATORY SYNCITIAL VIRUS (RSV) AND ITS POSSIBLE ASSOCIATION WITH *Clostridium difficile* INFECTIONS (CD)

Grilc Eva[1], Kastelec Damijana[2], Milavec Maja[1], Korošec Aleš,[1]Steiner Rihtar Saša[1], Sočan Maja[1]

[1]National Institute of Public Health (NIJZ), Ljubljana, Slovenia[2] Biotechnical Faculty Ljubljana Slovenia

Background and Aim: RSV and CD infections are more common in winter. Besides antibiotic use which influence the CD incidence the study in Canada showed that infections with RSV could impact incidence of CD (1). This fact is important as the global CD incidence is increasing and not all factors which influence it are well understood.

NIJZ and Biotechnical Faculty Ljubljana performed a pilot investigation to describe the time series of RSV and CD infections and antibiotics prescribed for respiratory infections during years 2012 to 2016 with the aim to evaluate the potential effect of RSV infections on CD infections. Since we have only aggregated data for RSV we performed only first step of investigation and described time series of RSV, antibiotic use and CD and cross correlation among them.

Methods: Data used for investigation were obtained from sentinel network for acute respiratory infections (RSV) and from surveillance databases on antibiotic use and CD infections in NIJZ from 2012 to 2016.

Weekly time series of CD infections, relative number of RSV infections and amount of four different antibiotics prescribed were examined to determine seasonal and trend component of each time series and cross-correlation analysis was performed to investigate if there is some correlation between CD infections and other four time series.

Results: Weekly time series of CD infections shows small positive trend component and even smaller seasonal component with period of 52 weeks (one year). The large part of variability of CD infections could be explained with random effect of weeks. The time series of relative RSV has evident seasonal component with the same period as CD with maximums during winter, and negative trend component. There is no important correlation between the CD and relative RSV time series even not between lagged time series (cross-correlation). We found that there exists some positive cross-correlation between CD infections and number of prescribed fluoroquinolones. Other two antibiotics (macrolides, beta lactam antibiotics) do not correlate with CD infections. There was negative correlation between CD and trimetophrim sulphametoxasole.

Conclusion: the analysis gave some preliminary data. Further studies which include data of RSV and CD infections with longer time series are needed to asses the possible impact of RSV on CD infections.

P90

EMERGENCE OF PREDOMINANTLY COMMUNITY-ASSOCIATED *Clostridium difficile* RT 012 IN WESTERN AUSTRALIA: RISK FACTORS AND RELATEDNESS TO STRAINS FROM ASIA

Lim Su-Chen[1], Dups Johanna[6], Knight Daniel[4], Bloomfield Lauren[3], Collins Deirdre[3], Foster Niki[5], Riley Thomas[1,2,3,4]

[1]School of Biomedical Sciences, The University of Western Australia, WA, Australia; [2]Department of Microbiology, PathWest Laboratory Medicine, Nedlands, WA, Australia; [3]School of Medical & Health Sciences, Edith Cowan University, Joondalup, WA, Australia; [4]School of Veterinary & Life Sciences, Murdoch University, Murdoch, WA, Australia; [5]OzFoodNet, Communicable Disease Control Directorate, Department of Health, Government of Western Australia, WA, Australia ; [6]National Centre for Epidemiology and Population Health, Australian National University

Background and aims: Since 2013, Western Australia (WA) has experienced an emergence of predominantly community-associated *Clostridium difficile* infection (CA-CDI) caused by ribotype (RT) 012. Recently, this strain was also identified as one the most prevalent RTs in Asia; accounting for nearly 20% of all CDI cases in Asia and associated with CA-CDI in China and South Korea.

Methods: A retrospective case-control study was carried out to determine the potential risk factors of CDI caused by RT 012 in WA compared to other RTs. Whole-genome sequencing (WGS) and high-resolution core genome phylogenetic analysis were performed on a small collection of 11 C. difficile RT 012 isolated from vegetables in WA in 2015 (n = 1), humans in Australia between 2013 and 2015 (n = 4) and humans in Asia in 2014 (n = 6).

Results: Eating raw non-organic home-grown vegetables and having contact with an individual living in a nursing home were associated with CDI caused by RT 012 (OR 16.50, 95% CI 1.09 – 250.18 for both). All sequenced RT 012 strains belonged to sequence type (ST) 54 and displayed allelic conservation in the seven housekeeping genes (adk1, atpA4, dxr7, glyA1, recA1, sodA3 and tpi3). Core genome single nucleotide variant (SNV) analysis revealed a clonal relationship between human strains from WA and Thailand (2 SNVs difference in core genome), suggestive of international transmission either from returned travellers and/or local foodborne transmission through food imported from Asia. This WA human strain was also more closely related to Asian strains (average 10.3 SNVs difference) than other sequenced human strains from WA (average 25 SNVs difference). The vegetable strain from WA was genetically very distant from all other sequenced RT 012 with an average SNVs difference of 884. Antimicrobial resistance (AMR) genes were identified in 72.7% of the isolates, 50% of which were considered multidrug resistant with AMR genes for tetracycline, aminoglycosides and macrolides-lincosamide-streptogramins.

Conclusions: To date, RT 012 has never been isolated from Australian livestock and is rare among WA food and environmental samples. Thus, local establishment and widespread dissemination of RT 012 in the community are unlikely. Collectively, these studies support the hypothesis that C. difficile RT 012 may be of Asian origin.

P91

WHOLE-GENOME SEQUENCING REVEALS POTENTIAL SPREAD OF *Clostridium difficile* BETWEEN HUMANS, FOODS AND THE ENVIRONMENT OF WESTERN AUSTRALIA

Lim Su-Chen[1], Knight Daniel[4], Foster Niki[5], Riley Thomas[1,2,3,4]

[1]School of Biomedical Sciences, The University of Western Australia, WA, Australia; [2]Department of Microbiology, PathWest Laboratory Medicine, Nedlands, WA, Australia; [3]School of Medical & Health Sciences, Edith Cowan University, Joondalup, WA, Australia; [4]School of Veterinary & Life Sciences, Murdoch University, Murdoch, WA, Australia; [5]OzFoodNet, Communicable Disease Control Directorate, Department of Health, Government of Western Australia, WA, Australia

Background and aims: Contaminated food and the environment are potential reservoirs for *Clostridium difficile* infection (CDI) in humans. Recently, *Clostridium difficile* ribotype (RT) 056, one of the most common RTs in Australian cattle and human infections, was isolated from vegetables and the environment in Western Australia (WA).

Methods: Whole-genome sequencing (WGS) and high-resolution core genome phylogenetic analysis were performed on a collection of 29 *Clostridium difficile* RT 056 isolated from humans between 2011 and 2015 (n = 21), vegetable in 2015 (n = 4) and the environment between 2007 and 2016 (n = 4) in WA.

Results: All sequenced RT 056 strains belonged to sequence type (ST) 34 and displayed allelic conservation in the seven housekeeping genes (adk1, atpA5, dxr7, glyA1, recA1, sodA3 and tpi1). Core genome single nucleotide variant (SNV) analysis found 14% of human strains showed a clonal relationship (≤ 2 SNVs difference) with one or more vegetable or environmental strains, consistent with recent foodborne and/or environmental transmission. Clones were isolated 2 – 3 years apart with 50% of the human cases occurring without recent healthcare exposure. The food and environmental *Clostridium difficile* were likely to be in a metabolically dormant spore form with a slower molecular clock compared to vegetative C. difficile. These findings suggest that over an extended period of time there have been persistent community reservoirs of C. difficile in vegetables and the environment in WA due to agricultural recycling of animal manure and human biosolids. Antimicrobial resistance (AMR) genotypes and phenotypes were largely in agreement, with resistance only observed in three human strains to clindamycin and erythromycin (ermB) and erythromycin-only with an unknown AMR gene.

Conclusions: This study supports the hypothesis that foodborne and environmental transmission of C. difficile occurs. On-going surveillance of human, animal, food and environmental C. difficile is needed to identify other potential reservoirs and reduce the overall burden of CDI.

P92

WHOLE-GENOME ANALYSIS OF *Clostridioides difficile* STRAINS ISOLATED FROM HORSES IN JAPAN

Hidekazu Niwa[1], Tsuyoshi Sekizuka[2], Makoto Kuroda[2], Eri Uchida[1], Yuta Kinoshita[1], Yoshinari Katayama[1], Mitsutoshi Senoh[3], Haru Kato[3]

[1] Equine Research Institute, Japan Racing Association, Japan; [2] Pathogen Genomics Center, National Institute of Infectious Diseases, Japan; [3] Department of Bacteriology II, National Institute of Infectious Diseases, Japan

Clostridioides difficile is recognized as an important cause of acute enterocolitis in horses. Some *Clostridium difficile* isolates we previously obtained from horses had identical PCR-ribotypes to those in humans, such as the hypervirulent RT 027 and 078. Recently, the possibility of *Clostridium difficile* as a potential zoonotic agent has been raised. We conducted whole-genome sequencing (WGS) analysis of equine *Clostridium difficile* isolates in Japan and compared the sequences with a number of other *Clostridium difficile* genome sequences.

Thirty-three *Clostridium difficile* isolates were obtained from horses suffering from intestinal disorders between May 2010 and July 2016. All of the isolates were toxin-producing strains. The isolates were sequenced on an Illumina NextSeq 500 sequencer, and 612 publicly available *Clostridium difficile* genome sequences were used as references. Computer-based multi-locus sequence typing (MLST), antimicrobial-resistant gene analysis, and core genome single nucleotide polymorphism (SNP)-based phylogenetic analysis were conducted on a Web-based genome analysis system (GenEpid-J).

The isolates were classified into 12 ribotypes and 13 MLST types. Although each ribotype of most of the isolates corresponded to each MLST type, a few ribotypes showed exceptions, especially RT 014. Core genome SNP-based phylogenetic analysis clustered the RT 078 isolates into 3 sublineages which corresponded to their geographical and temporal differences, and strongly suggested one of the lineages as the cause of a nosocomial outbreak in the equine hosptal. We could not distinguish the isolates of most of the ribotypes in horses from strains isolated from humans despite host and geographical differences. WGS analysis suggested the presence of a sublineage of RT 078 in Japanese horses associated with the nosocomial outbreak. It also revealed high genetic relatedness between equine and clinical isolates, which indicates the possibility of transmission of *Clostridium difficile* between horses and humans. However, the sources of *Clostridium difficile* in the Japanese equine population are still unknown. Therefore, continuous observation will be important from the perspective of the "One Health" concept.

P93

MOLECULAR CHARACTERISATION AND ANTIMICROBIAL RESISTANCE PATTERNS IN *Clostridium difficile* ISOLATED FROM THE ENVIRONMENT, HUMANS, AND OTHER ANIMAL SPECIES ORIGINATED FROM THE IBERIAN PENINSULA

Andrés-Lasheras Sara[1,2], Martín-Burriel Inma[3], Mainar-Jaime Raúl-Carlos[2], Sevilla Eloisa[2], Morales Mariano[2], Moreno Bernardino[2], Kuijper Ed[4], Chirino-Trejo Manuel[,5], Bolea Rosa[*,2]*
** These authors contributed equally to this work.*

[1]Agriculture and AgriFood Canada, Lethbridge Research and Development Centre, Lethbridge, Canada; [2]University of Zaragoza-CITA, Faculty of Veterinary Medicine, Zaragoza, Spain; [3]Universidad de Zaragoza-CITA, Laboratorio de Genética Bioquímica (LAGENBIO), Zaragoza, Spain; [4] Leiden University Medical Centre, Centre of Infectious Diseases, Leiden, The Netherlands; [5]University of Saskatchewan, Western College of Veterinary Medicine, Saskatoon, Canada.

Clostridium difficile is a microorganism that can infect several animal species among which are human beings. It is a spore-forming bacterium, thus it can be also isolated from different environmental sources. In the last years, the literature related with its presence in different animal species and environmental niches has grown, as the studies about the identification of close genetically related strains in humans and animals. Hence, it started to be considered a zoonotic agent. The aim of this study was to assess the presence of *Clostridium difficile* and its molecular and antimicrobial resistance diversity isolated from different sources located in the Iberian Peninsula. For this purpose, a total of 734 samples were analysed. From them, 40% originated from different animal species (faecal samples), 18.7% human beings (faecal samples), and 41.3% the environment (faecal and animal food samples). *Clostridium difficile* was isolated from 8.7% of the samples (46.9% from animals, 21.9% human beings, and 31.2% environment), being 76.6% of them toxigenic. A total of 22 and 3 PCR-ribotypes (RT) and toxinotypes were detected respectively, being RT078 the most common genotype found (25%). Only two ribotypes (078 and 010) were isolated from all kind of samples studied (animals, humans, environment), whereas the epidemic and hypervirulent RT027 was not detected in this study. The global resistance percentage observed to vancomycin, metronidazole and moxifloxacin was lower than to tetracycline, erythromycin and clindamycin. All the strains obtained in this work resulted sensible to vancomycin. Metronidazole resistant isolates were detected in animal and human samples. Non-toxigenic strains showed higher rates of resistance to metronidazole, erythromycin, clindamycin, and multidrug resistance (MDR) than toxigenic strains. In conclusion, the isolation of the same ribotypes from animals and people suggest an inter-species transmission or a common source of contamination. However, more discriminatory molecular techniques, as whole genome sequencing, are needed to elucidate its transmission routes in the community. The presence of *Clostridium difficile* strains resistant to metronidazole (stable) and the high proportion of MDR among non-toxigenic isolates highlight the importance of non-toxigenic strains as antibiotic-resistant determinants source.

P94

PREVALENCE AND CHARACTERIZATION OF *Clostridium difficile* FROM DOGS AND CATS IN KOREA

Ha-Young Kim[1], Jong Wan Kim[1], Chung-Hyun Kim[1], Yu-Ran Lee[1], Heejung Kim[2], ByungJae So[1]

[1]Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, Gimcheon 39660, Republic of Korea; [2]Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei Univeristy College of Medicine, Seoul 03722, Republic of Korea

Background and aims: *Clostridium difficile* has been recognized as an important emerging pathogen in both humans and different animal species. Animals are discussed as potential reservoirs and source of infection because of genetic overlap between animals and humans. This study was conducted to evaluate the prevalence of *Clostridium difficile* in dogs and cats and to characterize the isolates in Korea.

Methods: In a large-scale survey, we collected 793 fecal samples to evaluate the prevalence and characterize of *Clostridium difficile* in dogs and cats (688 dogs and 105 cats/ 265 shelter pets, 452 companion pets and 76 other dogs) from July 2016 – October 2017. PCR ribotyping, MLST, and PCR detection of toxin genes were used to characterize isolated *Clostridium difficile* strains. In addition, MIC was performed using E-test strips of ampicillin, cefoxitin, ciprofloxacin, erythromycin, metronidazole, moxifloxacin, tetracycline, and vancomycin.

Results: In total, *Clostridium difficile* was isolated from 156 out of 793 (19.7%) fecal samples; 149 from companion pets, six from shelter pets, and one from other dog. Among them, 7.4% (59/793) was toxigenic and 12.2% (97/793) was non-toxigenic strain. Most of the toxigenic strains were from companion pets (98.3%, 58/59). All 59 toxigenic strains were A+B+CDT- toxin gene profile, whereas PCR ribotypes and STs of toxigenic strains were variable. R106 (ST42) was the most prevalent ribotype (25.0%), followed by R014/020 (ST2), AB24 (ST129), AB25 (ST102), AB38 (ST36), R002 (ST8), R046 (ST35), R012 (ST54), R001 (ST3), R001 (ST29), R005 (ST63), R018 (ST17). All of the isolates were susceptible to metronidazole, vancomycin, and tetracycline, whereas they were resistant to cefoxitin and ciprofloxacin.

Conclusions: Based on the similarity between the ribotypes observed in this study and those described in humans in Korea, the zoonotic transmission for *Clostridium difficile* cannot be excluded. In addition, most of toxigenic *Clostridium difficile* (n = 58 out of 59) was isolated from companion pets, not outdoor pets, suggests that they might spread from humans to companion pets.

Clostridium difficile IN HONEY

P95

Joanna Wojtacka[1], Marta Sołtysiuk[1], Beata Wysok[1], Agnieszka Wiszniewska-Łaszczych[1], Joanna Szteyn[1], Robert Karczmarczyk[2], Małgorzata Gomółka-Pawlicka[1]

[1]University of Warmia and Mazury in Olsztyn, Faculty of Veterinary Medicine, Department of Veterinary Public Health, Poland; [2]Wrocław University of Environmental and Life Sciences, Faculty of Veterinary Medicine, Department of Epizootiology and Clinic of Bird and Exotic Animals, Poland

Background and aims: Bee honey is perceived as a functional food due to its unique composition, antimicrobial properties and bifidogenic effect. As a natural product that can not be subjected to any processing it is exposed to contamination with ubiquitous bacterial spores which are of environmental origin and persist in honey under typical storage conditions. The presence of *Clostridium difficile* spores in honey may pose a risk to induce CA-CDI especially to elderly who perceive honey as a natural remedy in many health problems including functional bowel abnormalities or natural intestine microflora support while using antimicrobials.

The aim of the study was to evaluate the prevalence of *Clostridium difficile* spores in directly sold honey produced in small apiaries usually located in the backyard of traditional farms in Poland.

Methods: A number of 80 samples of different types of honey (37 multifloral, 22 rape, 5 linden, 5 buckwheat, 4 honeydew, 3 nectar-honeydew, 3 acacia, 1 puffball) bought directly in the apiaries in Poland were analyzed for the presence of *Clostridium difficile*. All incubation procedures were performed in anaerobic conditions. The 25 g samples were pre-enriched in BHIS (6 days, 37oC). Sporulated forms were seeded on supplemented *Clostridium difficile* blood agar and incubated for 48 h at 37oC. Preliminary identification of *Clostridium difficile* strains was carried out with the use of rapid immunoenzimatic tests (GDH detection) on suspension of the colonies suspected according to McFarland 2.0. The detection of the tpi gene by PCR was also performed..

Results: Based on microbiological culture results (turbidity in liquid medium) 23 (28.75%) out of 80 honey samples were selected for culture on blood agar. Ground-glass colonies were noted on 10 plates. The presence of *Clostridium difficile* was not confirmed by immunoenzymatic rapid tests towards any sample selected. Neither PRC assay targeted to tpi gene showed positive samples.

Conclusions: This is the first report reviewing the possibility of honey contamination with *Clostridium difficile*. Polish honey produced in small apiaries (up to 20 hives) was free from *Clostridium difficile* spores which is of special importance due to the fact that so-called ecologic small apiaries promote their honey as part of healthy diet mainly to elderly and children.

Research funded by National Science Centre Poland, No DEC-2017/01/X/NZ9/00032
Supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015

P96

Clostridium difficile CONTAMINATION OF GERMAN RETAIL FOOD

Maurischat, S., Heise, J., Witt, P., Wichmann-Schauer, H.

German Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany

Clostridium (C.) difficile is a strictly anaerobic spore-former and well-known cause of healthcare-associated infections, primarily accompanied by an antibiotic therapy. Recently, reports are emerging that indicate a considerable proportion of hospitalized cases of *Clostridium difficile* infections (CDI) to be community-acquired. These cases differ from the classical risk population and can affect younger people or are independent from antibiotic treatments. Among different hypothesized infection routes, zoonotic transmissions might occur based on descriptions of phylogenetic relationships between human and animal isolates and varying prevalence in food worldwide.

From 2016 to 2018, we analysed food of animal (pork ground meat, n=148, and chicken meat, n=250) and non-animal origin (leaf salad, n=250) from German retail.

Samples were enriched in TPGY supplemented with taurocholate for 48–96 h followed by a second selective enrichment step using moxalactam-norfloxacin supplementation for another 48 h. Suspected *Clostridium difficile* colonies were isolated on ChromID *Clostridium difficile* agar (Biomerieux). Food samples were screened after the first enrichment and suspicious colonies were confirmed using real-time PCR.

Prior method validation with artificially contaminated salad and pork ground meat proved this method to be highly sensitive and specific. The detection limit was sufficient to detect even contaminations below one spore/gram food.

The results ranged from low but apparent *Clostridium difficile* contamination rates in pork ground meat (1.4%) and leaf salad (3%) to higher rates in chicken meat (15%). Most of the isolated strains were toxigenic carrying tcdA and tcdB genes. Furthermore, we also found RT078 strains carrying the binary toxin gene cdtA/B. Other PCR-ribotypes identified are mostly well-known for human CDI in Europe (e.g. RT002, RT014).

In conclusion, this is the first description of *Clostridium difficile* contamination of food products in Germany. Characterization and typing of these isolates reveals their similarity with endemic *Clostridium difficile* strains from human CDI and point towards their potential to induce a toxin-mediated diarrhea in humans. Further investigations have to be implemented to elucidate transmission pathways and the resulting relevance for human CDI.

ZERO PREVALENCE OF *Clostridium difficile* IN GERMAN WILD GAME

P97

Heise, J.[1], Gremse, C.[2], Jaeckel, C.[1], Maurischat, S.[1]

[1]German Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany; [2]German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Berlin, Germany

The strictly anaerobic spore-forming bacterium Clostridium (C.) difficile is a known pathogen of elder humans, mainly related with a hospitalization. Beside this, an increasing occurrence of *Clostridium difficile* infections (CDI) independent of hospital stays and age of the person was recorded within the last years. So far, the sources of an outpatient infection have not been identified, but a zoonotic transmission to humans based on direct contact with animals or the consumption of food is probable. *Clostridium difficile* was found in healthy, food-producing animals and it was possible to obtain closely related isolates from human and animal samples. Prevalence studies of wild animals are still limited, and there is no previous research on German wild game at all.

Here, we investigated in total 210 faecal samples of wild boars, roe deer, and red deer collected on ten different hunts in Brandenburg/Germany between November and December 2017. We used a selective enrichment followed by a species-specific real-time PCR assay for screening purposes and plating onto ChromID *Clostridium difficile* agar plates (Biomerieux) to identify *Clostridium difficile* in animal faeces. Suspicious colonies were confirmed by MALDI-ToF. Prior method optimization using artificial contaminated faeces proved the optimal enrichment length to be four days at 37°C under anaerobic conditions. A previous ethanol treatment was assessed to be not suitable as it indeed reduced the accompanying microbiota to a large extent, but also led to a strong reduction of one target strain.

The real-time PCR screening revealed all of the 210 stool samples of German wild game as *Clostridium difficile*-negative. This was confirmed by plating onto ChromID *Clostridium difficile* agar plates which resulted in no or atypical growth without any evidence of the occurrence of *Clostridium difficile*. The results were regardless of gender and age of the animals.

This first prevalence analysis of German wild game indicates no endemic colonization of wild animals by *Clostridium difficile* in Brandenburg/Germany as it is described for pets and food producing animals elsewhere. Further investigations especially on young wild animals are conceivable, because a higher prevalence in, for example, foals and piglets has been shown.

P98

ARE PET OWNERS AT INCREASED RISK OF *Clostridium difficile* INFECTION?

Rabold Denise[1], Espelage Werner[2], Abu-Sin Muna[2], Eckmanns Tim[2], Möbius Nadine[3], Hille Katja[3], Neubauer Heinrich[4], Schneeberg Alexander[4*], Wieler Lothar H.[1,2], Seyboldt Christian[4*], Lübke-Becker Antina[1*]

[1]Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; [2]Robert Koch Institute, Berlin, Germany; [3]Department of Biometry, Epidemiology and Information Processing, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany; [4]Institute of Bacterial Infections and Zoonoses, Federal Research Institute for Animal Health (Friedrich-Loeffler-Institut), Jena, Germany; *current address: Landestierärztekammer Thüringen, Erfurt, Germany
∗ These authors contributed equally to this work.

It can be assumed that about one quarter of the human infections with Clostridioides (C.) difficile occur within the community, yet, this pathogen is known to be the primary cause of antibiotic- and hospital-associated diarrhoea. However, the source of infection has not yet been determined. The role of animals for community-acquired infections has long been discussed and, within this context, the findings on *Clostridium difficile* in various animal species and the overlap between ribotypes are of particular concern. Nonetheless, especially epidemiological data on *Clostridium difficile* in companion animals is scarce.

This study aimed to collect data on the occurrence and genotypic variation of *Clostridium difficile* in dogs, cats and their owners in Germany and to define risk factors associated with faecal shedding of this pathogen. From July 2012 to August 2013, a Germany-wide survey was conducted sampling companion animals and their owners. Capillary gel electrophoresis based PCR ribotyping, Multilocus VNTR Analysis (MLVA), and PCR detection of toxin genes were used to characterize isolated *Clostridium difficile* strains. Statistical and phylogenetic analyses were performed using STATA® and BioNumerics.

In total, 1,447 faecal samples were collected throughout Germany with 1,418 samples from 415 different households meeting the inclusion criteria. The isolation rates for companion animals and their owners were similarly low with 3.0% (25/840) and 2.9% (17/578), respectively. Typing revealed twelve resp. eight different PCR ribotypes in isolates of human resp. animal origin, with ribotypes 014/0, 010 and 078 prevalent in both groups. The potentially highly pathogenic human ribotypes 027 and 078 were also isolated in dogs. Within two households identical ribotypes were isolated from two partner animals, whereas no *Clostridium difficile* pair from owner and pet sharing the same household could be detected. Nevertheless, the results of the epidemiological risk assessment and phylogenetic analysis of isolated ribotypes support the hypothesis of a zoonotic potential.

To conclude, C. difficile isolation rates are low in companion animals and their owners in Germany. However, molecular characterization and epidemiological analysis revealed that the zoonotic potential of *Clostridium difficile* associated with dogs and cats within the community is low but cannot be excluded.

P99

HIGH PREVALENCE OF *Clostridium difficile* IN SOIL, MULCH AND LAWN SAMPLES FROM WESTERN AUSTRALIAN (WA) HOSPITALS

Perumalsamy Sicilia[1], *Putsathit Papanin* [2] *and Riley Thomas V.*[1,2,3,4]

[1]*The University of Western Australia, School of Biomedical Sciences, Queen Elizabeth II Medical Centre, Nedlands, Western Australia;* [2]*School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, Australia;* [3]*PathWest Laboratory Medicine, Department of Microbiology, Queen Elizabeth II Medical Centre, Nedlands, Western Australia, Australia;* [4]*School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia.*

Background and aims: *Clostridium difficile* infections (CDI) have been increasing in people without hospital contact, despite remaining a major hospital pathogen. Non-human reservoirs of CDI include animals, food and the environment. Toxigenic *Clostridium difficile* strains have been found also on soles of shoes and boots, raising the possibility of introducing contaminated soil from outside hospital environments. Herein, we report the prevalence of *Clostridium difficile* in the immediate outdoor environment of different hospitals, providing further insight into potential sources of community acquired infections.

Methods: In 2018, a total of 146 samples consisting of soil, mulch, lawn and sand were collected from the outdoor surroundings of 4 different old and new hospitals in Perth, WA. All samples were incubated in *Clostridium difficile* selective enrichment broth (BHIB supplemented with cycloserine and cefoxitin) for at least 5 days, followed by alcohol shock and culture on *Clostridium difficile* ChromID. PCR toxin gene profiling and ribotyping was performed, and PCR ribotypes (RTs) identified by comparing banding patterns to our reference library.

Results: *Clostridium difficile* was isolated from 86 out of 145 (59.3%) samples. Overall, 28.8% (28/97) of the isolates were toxigenic (A+B+CDT-, n=22; A-B+CDT-, n=5; A+B-CDT-, n=1). A totalof 24 RTs were novel non-toxigenic strains followed by RT 014/020 (A+B+CDT-), 010 (A-B-CDT-), QX189 (A-B-CDT-), 298-like (A-B-CDT-), QX284 (A-B-CDT-), QX150 (A+B+CDT-), and 103 (A+B+CDT-). Interestingly, RT 017, a strain that is endemic to the Asia-Pacific region, was also found from a newly laid lawn around one of the older hospitals.

Conclusions: The presence of highly diverse strains in hospitalised patients suggests the possibility of patients acquiring infections from sources/reservoirs external to the hospital. This is the first study to identify *Clostridium difficile* in outdoor environment of various hospitals. Even though *Clostridium difficile* is commonly found ubiquitously in soil, the presence of toxigenic strains especially RT014/020, RT103 and the Asian strain of much interest, RT017, is of concern. The actual risk of disease is unclear. Additional studies are required to determine the prevalence of *Clostridium difficile* on shoes of healthcare staff in WA that may facilitate the introduction of different strains from community sources into hospitals.

P100

DOES NAAT TEST USE INCREASE THE RATE OF *Clostridium difficile* INFECTION DIAGNOSIS?

Guido Granata[1], Silvia D'Arezzo[2], Genoveffa Francesca Sapia[3], Chiara De Giuli[2], Carolina Venditti[2], Marcello Meledandri[3], Nicola Petrosillo[1], Maria Adriana Cataldo[1]

[1]National Institute for Infectious Diseases "Lazzaro Spallanzani", Clinical and Clinical Research Department, Rome, Italy; [2]National Institute for Infectious Diseases "Lazzaro Spallanzani", Microbiology Laboratory, Rome, Italy; [3]San Filippo Neri Hospital, Microbiology Laboratory, Rome, Italy.

Background and aims: The rate of under-diagnosis of *Clostridium difficile* infection (CDI) is a major issue affecting CDI management and it is relevant from both an epidemiological and a clinical point of view. The aim of our study was to evaluate different diagnostic approaches for CDI to lessen the risk of under-diagnosis.

Methods: All adult patients admitted to two acute care hospitals and presenting diarrhea were prospectively enrolled in the study. Demographic data; time of diarrhea onset; admissions in healthcare settings, exposure to healthcare procedures and to antibiotics in the previous 3 months were ascertained.

GDH and toxin A and B EIA (ToxA/B), nucleic acid amplification test (NAAT) and toxigenic culture (TC) were performed on each collected stool samples. CDI was diagnosed considering both microbiological results and clinical information.

Results: During the study period, 546 hospitalized patients with diarrhea were enrolled in the study. Overall 131 cases of CDI were diagnosed. In 61% of cases (80) both GDH and ToxA/B were positive, in 20% (26) and 19% (25) of cases NAAT and TC test results were positive, respectively.

In 3% of patients with GDH and ToxA/B test negative (4/131) we were able to diagnose CDI by NAAT and by clinical characteristics (other causes of diarrhea were also excluded). We calculated that NAAT could diagnose 1 CDI per 100 patients with diarrhea. After analyzing subgroup of patients with higher risk of CDI and GDH/ToxA/B test negative, we calculated that 52 patients with HCA diarrhea should be tested by NAAT to diagnose 1 CDI; 45 patients with previous exposure to antibiotics and 34 patients with HCA diarrhea and previous antibiotic exposure need to be tested by NAAT to diagnose 1 CDI.

Conclusions: The use of NAAT in patients with suspicion of CDI may be helpful in increasing the rate of CDI diagnosis also in cases whose stool samples resulted both GDH and ToxA/B negative. The number of NAAT to be performed to increase the rate of CDI diagnosis is not high, consequently a significant impact on costs and work load is not expected.

P101

CAN SHOE SOLES CONTRIBUTE TO *Clostridium difficile* DISSEMINATION IN HOSPITAL ENVIRONMENT?

Sandra Janezic[1,2], *Ines Blazevic*[2], *David Eyre*[3,4], *Aleksander Kocuvan*[2], *Maja Rupnik*[1,2]

[1]*National Laboratory for Health, Environment and Food, Maribor, Slovenia;* [2]*University of Maribor, Faculty of Medicine, Maribor, Slovenia,* [3]*Nuffield Department of Medicine, University of Oxford, Oxford, UK,* [4]*Big Data Institute, University of Oxford, Oxford, UK*

Shoe soles are shown to have a dense pathogenic load and shoes can be a reservoir and a vector that spreads bacteria through different environments, posing threat to human infections (PMID: 27495010). In this study, we aimed to evaluate the presence of *Clostridium difficile* on shoe soles to explore their possible contribution to the dissemination of *Clostridium difficile* spores in hospital environment.

Altogether 55 shoe sole swabs were taken from health-care staff (physicians, nurses, cleaning staff and paramedics), medical students and a visitor from different departments within a single teaching hospital. Sampling was performed in May, July and August in 2014.

Almost two-thirds (62 %) of shoe swabs were positive for *Clostridium difficile*. Eleven different PCR ribotypes were identified, with most common being PCR ribotypes 010 (n=8), 027 (n=8), 014/020 (n=7), 018 (n=5) which are also ribotypes that are commonly isolated from patients. Fourteen shoe isolates and 39 matching (temporal and spatial) clinical isolates of PCR ribotypes 027 (n=31), 014/020 (n=15) and 002 (n=7) were selected for whole genome sequencing and high-resolution single nucleotide variant (SNV) analysis to determine their relatedness on a whole genome level. With core genome SNV analysis 12 out of 14 (86 %) of shoe sole isolates had $\leq$ 2 SNVs difference from one or more human clinical isolates which is consistent with recent transmission. The remaining two shoe isolates, both of PCR ribotype 014/020, were genetically distinct, had 10 or more SNVs compared to clinical isolates. Genetically related pairs of clinical and shoe strains were isolated from 3 days to 20 weeks apart and were found both within the same departments as well as in different departments. We demonstrated a high rate of *Clostridium difficile* contamination of shoe soles in hospital environment and high prevalence of *Clostridium difficile* PCR ribotypes that are commonly isolated from humans. This study also shows that shoe soles could play an important part in the dissemination of *Clostridium difficile* spores in hospital environment.

P102

WHOLE GENOME ANALYSIS OF *Clostridium difficile* PCR RIBOTYPE 150 ISOLATED FROM HUMANS, ANIMALS, ENVIRONMENT AND FOOD

Sandra Janezic[1,2], David Eyre[3,4], Tanja Vrabic[1,2], Maja Rupnik[1,2]

[1]National Laboratory for Health, Environment and Food, Maribor, Slovenia; [2]University of Maribor, Faculty of Medicine, Maribor, Slovenia, [3]Nuffield Department of Medicine, University of Oxford, Oxford, UK, [4]Big Data Institute, University of Oxford, Oxford, UK

Clostridium difficile PCR ribotype 150 is among the six most common PCR ribotypes isolated from humans, animals and environmental samples in Slovenia and was also the third most common toxigenic PCR ribotype isolated from potato surfaces in our recent study. The aim of the present study was to determine relatedness of ribotype 150 isolates from different sources on a whole genome level.

For a screening purpose, we first performed a pulsed field gel electrophoresis (PFGE) on a collection of 53 *Clostridium difficile* PCR ribotype 150 strains, isolated in Slovenia from humans (n=36), animals (n=11), environment (n=2) and potatoes (n=4) between 2011 and 2016. Sixteen isolates originating from all four reservoirs that had identical PFGE profile were then subjected to whole genome sequencing and high-resolution single nucleotide variant (SNV) analysis to confirm their relatedness. In silico MLST typing was performed with SeqSphere+ (Ridom, Germany).

All 16 sequenced PCR ribotype 150 strains belonged to MLST sequence type ST92. Three potato isolates had only app. 3.6 Mb consensus base count and were excluded from the final comparison. SNV analysis of the core genome of the remaining 13 successfully sequenced isolates showed clonal relationship between four human and a single potato isolate, which is consistent with recent transmission from a common source. The human strains originated from a single diagnostics laboratory and were isolated between December 2014 and October 2015, and the potato strain was isolated in March 2016. In addition, all potato strains were analyzed despite the lower quality of sequences and were also shown to be genetically related ($\leq$2 SNV differences). The four potato strains were isolated from three different samples, bought in three different local supermarkets, one was of Slovenian origin and two were imported from Cyprus and Egypt.

Similar as other studies we have shown the existence of clonally related strains spanning over long time intervals. Contamination point of potatoes is not clear, but our results suggest that they could be a possible way of introduction of *Clostridium difficile* in households and could also contribute to long distance dissemination of spores.

POTATOES SAMPLED ACROSS EUROPE ARE COMMONLY CONTAMINATED WITH *Clostridium difficile*

P103

Tkalec Valerija[1], Skok Barbara[1], Janezic Sandra[1,2], Rupnik Maja[1,2]

[1]National Laboratory for Health, Environment and Food, Centre for Microbiology, Maribor, Slovenia; [2]University of Maribor, Faculty of Medicine, Maribor, Slovenia

Foodborne transmissions have been considered as one of potential infection routes of *Clostridium difficile*. The bacterium has been detected in diverse range of foods, including meat, seafood and vegetables, however, studies on food, especially vegetables are limited. Here we present the prevalence of *Clostridium difficile* on potatoes collected in 15 different countries across the Europe.

A total of 242 potato swabs or samples sold in European retail stores or vegetable markets were collected between June 2015 and July 2018 from 15 countries. In general, a single sample included three potatoes swabbed with a sterile sponge. Sponge was incubated anaerobically for 5 to 7 days in BHIST selective broth (BHI (Biolife) supplemented with sodium taurocholate (0,1%), yeast extract (0,5%), 0,05% L-cysteine and *Clostridium difficile* selective supplement (Oxoid)), followed by alcohol shock and plating onto chromogenic selective plates (chromID C. difficile agar (bioMerieux)). Up to 12 presumptive colonies were subcultured on 5% horse blood agar (COH, bioMerieux). Identification was confirmed by mass spectrometry (MALDI Biotyper System; Bruker). All isolates were characterized by PCR ribotyping and one representative PCR ribotype from each sample was toxinotyped

.

Clostridium difficile was isolated from 72 out of 242 (29,8 %) samples. Percentage of *Clostridium difficile* positive samples ranged from 6,7 % and 8,3 % (UK and Austria, respectively) to 100,0 % (Romania, Bosnia and Herzegovina). All tested samples were negative only from one country (Slovak Republic). The information on potato origin was also collected and one third of *Clostridium difficile* positive potato samples was imported from foreign countries of three different continents (America, Africa, Europe).

Altogether 671 of isolates were obtained. Up to date, 169 were fully characterized and distributed into 30 different PCR ribotypes. Fourteen of those were toxigenic and PCR ribotypes 014/020, 053 (nontoxigenic) and 126 were most common. The majority of 30 PCR ribotypes found on potatoes have previously been reported in humans, animals, soil or water.

Our results show that large proportion of potatoes sold across the Europe is contaminated with *Clostridium difficile*. Although the exact point of contamination is not clear, the results also suggests that potatoes may represent a transnational and transcontinental way of *Clostridium difficile* spread.

P104

OBESITY-ASSOCIATED GUT MICROBIOTA ENHANCES *Clostridium difficile* INFECTION IN MICE

Shinsmon Jose[1], Anindita Mukherjee[1], Jianli Xue[1], Maria E. Moreno-Fernandez[2,4], Maneesh Chidambaram[1], Olivia Horrigan[1], Senad Divanovic[2,4], and Rajat Madan[1,3]

[1]Division of Infectious Diseases, Department of Internal Medicine, University of Cincinnati. Cincinnati, OH 45267. [2]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45220. Divisions of [3]Gastroenterology, Hepatology and Nutrition, and [4]Immunobiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229.

Background and Aims: *Clostridium difficile* is the leading cause of nosocomial infections in the U.S. Obesity, which is a modern-day epidemic, increases the risk of acquiring *Clostridium difficile* and is also associated with clinically severe *Clostridium difficile* infection (CDI). However, the mechanism(s) that increase CDI susceptibility in obese individuals and lead to worse clinical disease remain unknown.

Methods: We established a novel animal model of CDI in obesity by coupling a mouse model of high fat diet (HFD)-induced obesity with CDI. Obese and control (non-obese) mice pre-treated with antibiotics were challenged with *Clostridium difficile* spores by oral gavage, and disease parameters such as weight loss, diarrhea, tissue damage and bacterial burden were studied.

Results: We show that compared to control mice, obese mice had longer duration of clinical disease (weight loss and diarrhea), inflammation and colonic tissue damage. Worse clinical disease in obese mice correlates with persistence of both *Clostridium difficile* pathogen and toxins. During the early stages of infection, obese mice had lower toxin levels despite similar overall pathogen load, but the clearance of *Clostridium difficile* bacteria and toxins was delayed in obese mice. Host gut microbiota and metabolic environment can influence *Clostridium difficile* lifecycle (sporulation and germination). In fact, transfer of microbiota from obese mice increased diarrhea and mortality in control mice after CDI, and obese microbiota significantly enhanced *Clostridium difficile* spore germination in vitro compared to microbiota from lean controls.

Conclusions: Overall our data indicate that obesity-associated changes in commensal bacteria could alter dynamics of the *Clostridium difficile* life cycle and thus impact clinical disease.

P105

VARIOUS WAYS OF INTERACTIONS BETWEEN CLOSTRIDIUM DIFFICILE AND GUT MICROBIOTA

Horvat Sabina[1], Rupnik Maja[1,2]

[1]University of Maribor, Faculty of Medicine, Maribor, Slovenia; [2]National Laboratory for Health, Environment and Food, Centre for Microbiology, Maribor, Slovenia;

Clostridium difficile is an intestinal pathogen typically associated with dysbalanced gut microbiota. For this reason a considerable amount of research is focused on the associations between *Clostridium difficile* and gut microbiota and the underlying mechanisms.

Using a simple in vitro batch model we have previously shown that interactions between *Clostridium difficile* and microbiota are bidirectional. *Clostridium difficile* vegetative cells or conditioned media had influenced the diversity and composition of fecal microbiota. Adult dysbalanced microbiota showed different changes as adult healthy microbiota. Changes in microbiota composition were specific and similar to those observed in CDI patients, suggesting that dysbiosis initially caused by e.g. antibiotics and predisposing to CDI, is to some extent maintained by *Clostridium difficile* during and after the infection.

In the case of microbiota effects on *Clostridium difficile* we have shown that growth is strain dependent, while all strains showed higher sporulation frequency in the co-culture with dysbiotic fecal microbiota.

The aim of our study presented here was to compare the impact of *Clostridium difficile* vegetative cells and *Clostridium difficile* conditioned medium on gut microbiota of infants under 2 years of age in in vitro model. Six different strains belonging to ribotypes 027, 078 and 176 were used. Five out of six tested strains grew significantly better in control samples (i.e. growth medium only) than in co-cultures with infant microbiota. All six strains formed higher percentage of spores in co-culture with infant microbiota (20 - 57 %), while in control samples spore percentage was lower than 8 %.

Cultivation of infant fecal microbiota in the presence of vegetative cells or in the presence of conditioned medium decreased the diversity and significant differences ($p < 0.05$) were observed for Bacteroidetes, Firmicutes and Proteobacteria phyla.

The results indicate that *Clostridium difficile* is able to affect the infant microbiota. In addition, the high amount of *Clostridium difficile* spores present in co-cultures with microbiota could explain the high rate of asymptomatic carriage observed in infants.

P106

HOSPITAL COST SAVINGS USING ULTRASENSITIVE SINGLE MOLECULE COUNTING FOR DETECTION OF CLOSTRIDIUM DIFFICILE TOXINS A AND B

Bijlani Akash[1], Estis Joel[2], Sandlund Johanna[2]

[1]Strategy Access Solutions, San Carlos, California; [2]Singulex, Alameda, California

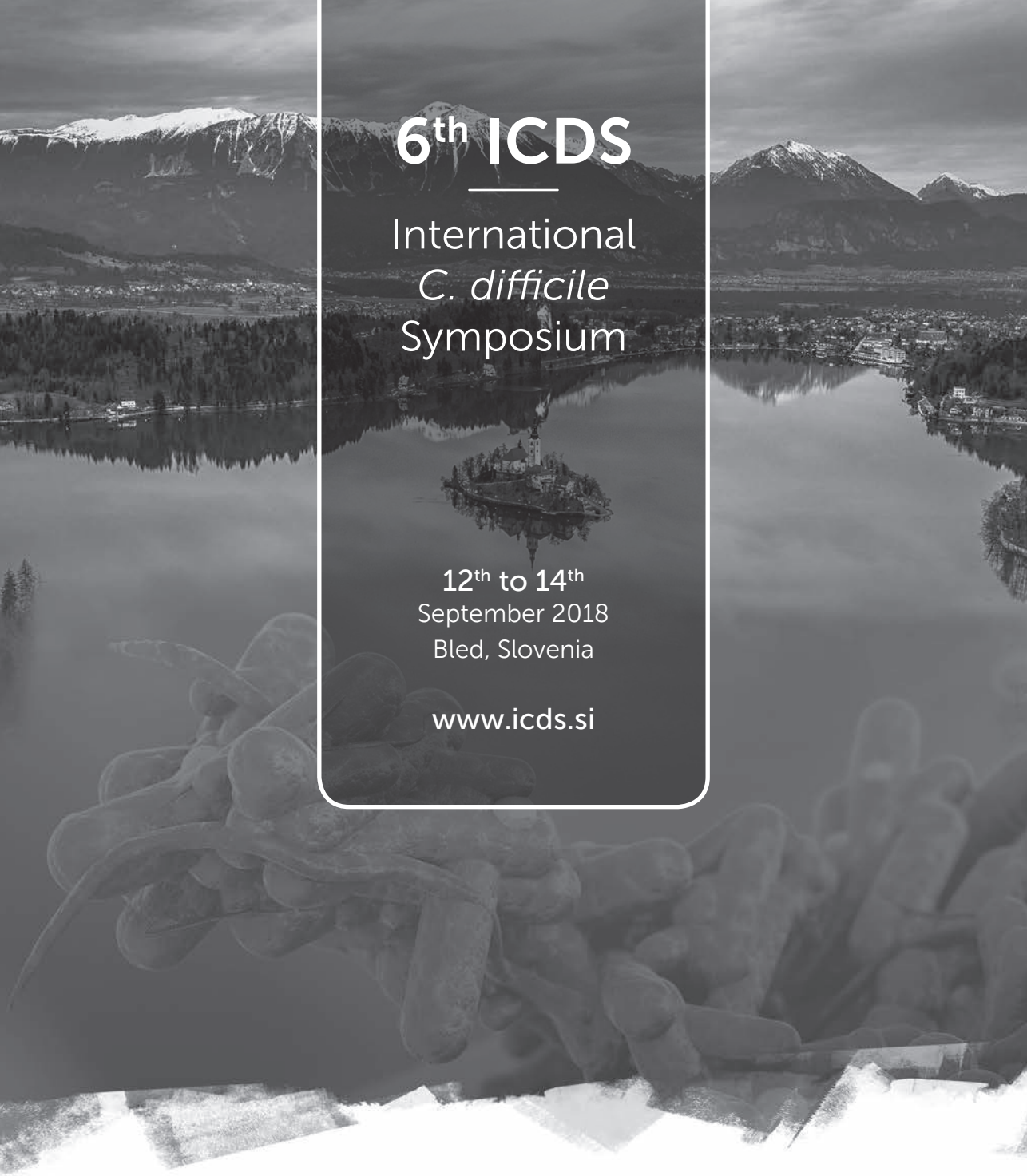
Background and Aims
Enzyme immunoassays (EIAs) for Clostridioides difficile (formerly Clostridium difficile) toxins have low sensitivity and lead to missed cases of C. difficile infection (CDI). With the introduction of nucleic acid amplification tests (NAATs), which cannot differentiate between colonized individuals and CDI patients, the CDI incidence at many institutions has increased. Yearly CDI-attributable healthcare costs in the U.S are up to $4.8 billion., and overdiagnosis may lead to additional hospital and payer cost. The Singulex Clarity® C. diff toxins A/B assay, in development for the Singulex Clarity system and using Single Molecule Counting technology, was designed to provide a highly sensitive, specific, and rapid detection of C. difficile toxins A and B in stool. The aim of this study was to understand the cost savings, in a U.S. context, of the Clarity C. diff toxins A/B assay compared to 4 other current CDI lab assays.

Methods
An economic model was developed to describe annual hospital cost savings, in a U.S. context, based on differences in sensitivity, specificity, and associated clinical outcomes of the Clarity assay compared to NAAT, GDH-and-toxin EIA, and multistep algorithms (GDH-and-toxin EIA followed by NAAT, and NAAT followed by toxin EIA if NAAT+). Key cost-saving measures included decreases in 1) preemptive isolation, 2) missed cases, 3) additional length of stay (LOS), 4) medication cost, 5) CDI hospital readmissions, and 6) CMS penalties.

Results
Across all cost saving categories, the biggest drivers were 1) decreasing additional LOS due to improved specificity and diagnosis of true CDI, and 2) reducing vancomycin prescribed for CDI-negative cases. Depending on the type(s) of current CDI lab tests and whether the hospital was/was not penalized by the national hospital-acquired conditions reduction program or value-based purchasing, the annual mean cost savings ranged from $150,000-$317,000 per institution. The most economic benefit was observed comparing the Clarity assay with GDH-and-toxin EIA and a multistep algorithm where NAAT is followed by toxin EIA if NAAT+.

Conclusions
With high sensitivity, specificity and a rapid turnaround time compared to current CDI test options, use of the Singulex C. diff toxins A/B assay may result in significant hospital cost savings.

P2



ICDS PARTICIPANTS

Participant	Country	Institution	Presentation
Aira Gomez, Andrea	Spain	IDIBAPS	P71
Akrich, Brune	France	MSD France	
Alibert, Patrice	Belgium	GenePOC	P62
Allavoine, Thierry	France	MSD-France	
Alves Feliciano, Carolina	France	Institut Pasteur	P43, P46
Androga, Grace	Australia	University of Western Australia	P18
Angulo, Fred	USA	Pfizer Vaccines	
Anttila, Veli-Jukka	Finland	Helsinki University Hospital	P82
Avberšek, Jana	Slovenia	University of Ljubljana, Veterinary Faculty	P61
Baines, Simon	United Kingdon	University of Hertfordshire	P5
Bajaj-Elliott, Mona	United Kingdom	University College London	P63
Barbut, Frédéric	France	Hopital saint antoine	P66, P67
Barranco, Carlos	Finland	ArcDia International Oy Ltd	
Barwinska-Sendra, Anna	United Kingdom	Newcastle University	P20, P27
Basseres, Eugenie	USA	University of Houston	P53
Beigot Glaser, Sara	Slovenia	National Laboratory of Health, Environment and Food	
Berce, Ingrid	Slovenia	National Laboratory of Health, Environment and Food	
Bernet-Camard, Marie-Françoise	France	Université Paris Sud	
Bijlani, Akash	USA	Strategy Access Solutions	P106
Blount, Ken	USA	Rebiotix Inc	P56
Boekhoud, Ilse	The Netherlands	Leiden University Medical Centre	INV17, P28, P59
Bohne, Wolfgang	Germany	Institute for Medical Microbiology	P26
Bolea, Rosa	Spain	University of Zaragoza	P59, P93
Britton, Robert	USA	Baylor College of Medicine	INV1, P13
Buckley, Tony	United Kingdom	University of Leeds	P8, P9, P10
Busti, Elena	Italy	Meridian Bioscience	
Camacho, Adrian	Mexico	Pfizer	
Cammarota, Giovanni	Italy	Fondazione Policlinico Gemelli	INV4
Chapman, Sarah	United Kingdom	University of Nottingham	P54

Participant	Country	Institution	Presentation
Chilton, Caroline	United Kingdom	University of Leeds	OP4, P8, P25
Coles, David	USA	Pfizer	
Collins, Deirdre	Australia	Edith Cowan University	P86, P90
Connor, Mairead	Northern Ireland	Queen's University Belfast	P81
Corver, Jeroen	The Netherlands	Leiden University Medical Center	INV17, P29, P30
Cottam, James	France	Cepheid	
Coullon, Heloise	France	Université Paris Sud - Faculté de Pharmacie	OP16
Crobach, Monique	The Netherlands	Leiden University Medical Centre	INV13, OP7
Cuenot, Elodie	France	Institut Pasteur	OP15
Curcio, Daniel	Argentina	Pfizer SRL	
Davies, Kerrie	United Kingdom	University of Leeds	INV14
Dawson, Lisa	United Kingdom	LSHTM	P17, P38
de Castro Brito, Gerly Anne	Brazil	Federal University of Ceará	P21, P22, P36
Delk, Trudi	USA	Synthetic Biologics, Inc.	P76
Delmée, Michel	Belgium	Université catholique de Louvain	P64
Dong, Danfeng	China	Shanghai Jiaotong University School of Medicine	P6
Donskey, Curtis	USA	Cleveland VA Medical Center	INV12
Dorr, Mary Beth	USA	Merck & Co., Inc.	OP1, OP6, P1
Dubberke, Erik	USA	Washington University School of Medicine	P56, P80
Dubischar, Katrin	Austria	Valneva Austria GmbH	
Duperchy, Esther	United Kingdom	Biotech	P52
Dupuy, Bruno	France	Institut pasteur	OP5, OP15, P40, P43, P45, P46
Elfassy, Arik	USA	Pfizer	P70
Etifa, Perezimor	United kingdom	University of Hertfordshire	P5
Externest, Doerthe	USA	Pfizer, Inc.	

Participant	Country	Institution	Presentation
Eyre, David	United Kingdom	University of Oxford	INV16, P101, P102
Fang, Ferric	USA	University of Washington	INV5
Fernandes, Nadia	United Kingdom	University of Sheffield	P33
Fortier, Louis-Charles	Canada	Université de Sherbrooke	INV9, P23
Garneau, Julian	Canada	Université de Sherbrooke	P23
Gerding, Dale	USA	Hines VA Hospital	P14, P55
Germain, Sophie	Belgium	GSK vaccines	
Girbau Aguilar, Javier	USA	Merck Sharp & Dohme Corp.	
Goering, Richard	USA	Creighton University School of Medicine	P79
Golding, George	Canada	Public Health Agency of Canada	OP9
Govoni, Gregory	USA	SciBac	P11
Granata, Guido	Italy	I.R.C.C.S. LAZZARO SPALLANZANI	P100
Grilc, Eva	Slovenia	National Institute of Public Health	P89
Grimes, Rachel	Ireland	Trinity College Dublin	
Groß, Uwe	Germany	University Medical Center Göttingen	P26
Halevi, Shiraz	Israel	Sheba Medical Center	
Harris, Hannah	United Kingdom	University of Leeds	P8, P25
Harrison, Mark	United Kingdom	London School of Hygiene and Tropical Medicine	P38
Haupt, Richard	USA	Pfizer Vaccines	
Heger, Florian	Austria	Austrian Agency for Health and Food Safety Ltd.	
Heidebrecht, Hans-Jürgen	Germany	Technical University of Munich	OP3
Ho, Jeffery	Hong Kong SAR	The Chinese University of Hong Kong	
Hong, Stacey	Australia	University of Western Australia	P19
Horvat, Nika	Slovenia	National Laboratory of Health, Environment and Food	
Imwattana, Korakrit	Australia	University of Western Australia	
Indra, Alexander	Austria	AGES IMED	
Ingebretsen, André	Norway	Oslo University Hospital	
Ingle, Patrick	United Kingdom	University of Nottingham	P39
Janezic, Sandra	Slovenia	National Laboratory for Health, Environment and Food	P101, P102, P103

Participant	Country	Institution	Presentation
Janoir, Claire	France	University Paris Sud	INV8, OP15, OP16, P2, P12
Johnson, Stuart	USA	Loyola University Medical Center	INV3, P14, P55
Jones, Hal	USA	Pfizer	P70
Jose, Shinsmon	USA	University of Cincinnati	P104
Juul, Frederik Emil	Norway	Oslo University Hospital	
Kato, Haru	Japan	National Institute of Infectious Diseases	P80, P83, P92
Kelly, Abigail	United Kingdom	Newcastle University	P24
Kim, Ha-Young	Republic of Korea	Animal and Plant Quarantine Agency	P68, P94
Kim, Heejung	Republic of Korea	Yonsei University Yongin Severance Hospital	P85, P94
Kim, Jong Wan	Republic of Korea	Animal and Plant Quarantine Agency	P68, P94
Kint, Nicolas	France	Institut Pasteur	P43, P46
Kitchin, Nicholas	United Kingdom	Pfizer Limited	
Knight, Dan	Australia	Murdoch University	INV15, P18, P19, P50, P90, P91
Kobeissy, Hussein	France	EA4043 Unité Bactéries Pathogène et santé	
Kocuvan, Aleksander	Slovenia	University of Maribor, Faculty of Medicine	P101
Kokai-Kun, John	USA	Synthetic Biologics, Inc.	OP2
Kong, Ka Yi	Hong Kong SAR	The Chinese University of Hong Kong	P15
Koskinen, Juha M.	Finland	University of Turku, Faculty of Medicine	P65, P69
Krutova, Marcela	Czech Republic	Charles University in Prague and Motol University Hospital	P73, P74
Kuijper, Ed J.	The Netherlands	Leiden University Medical Centre	INV17, OP7, P28, P30, P51, P59, P93
Le Monnier, Alban	France	GH Paris Saint-Joseph	P2, P3
Leite Costa, Cecília	Brazil	Federal University of Ceará	P36
Lejko Zupanc, Tatjana	Slovenia	University Medical Centre Ljubljana	

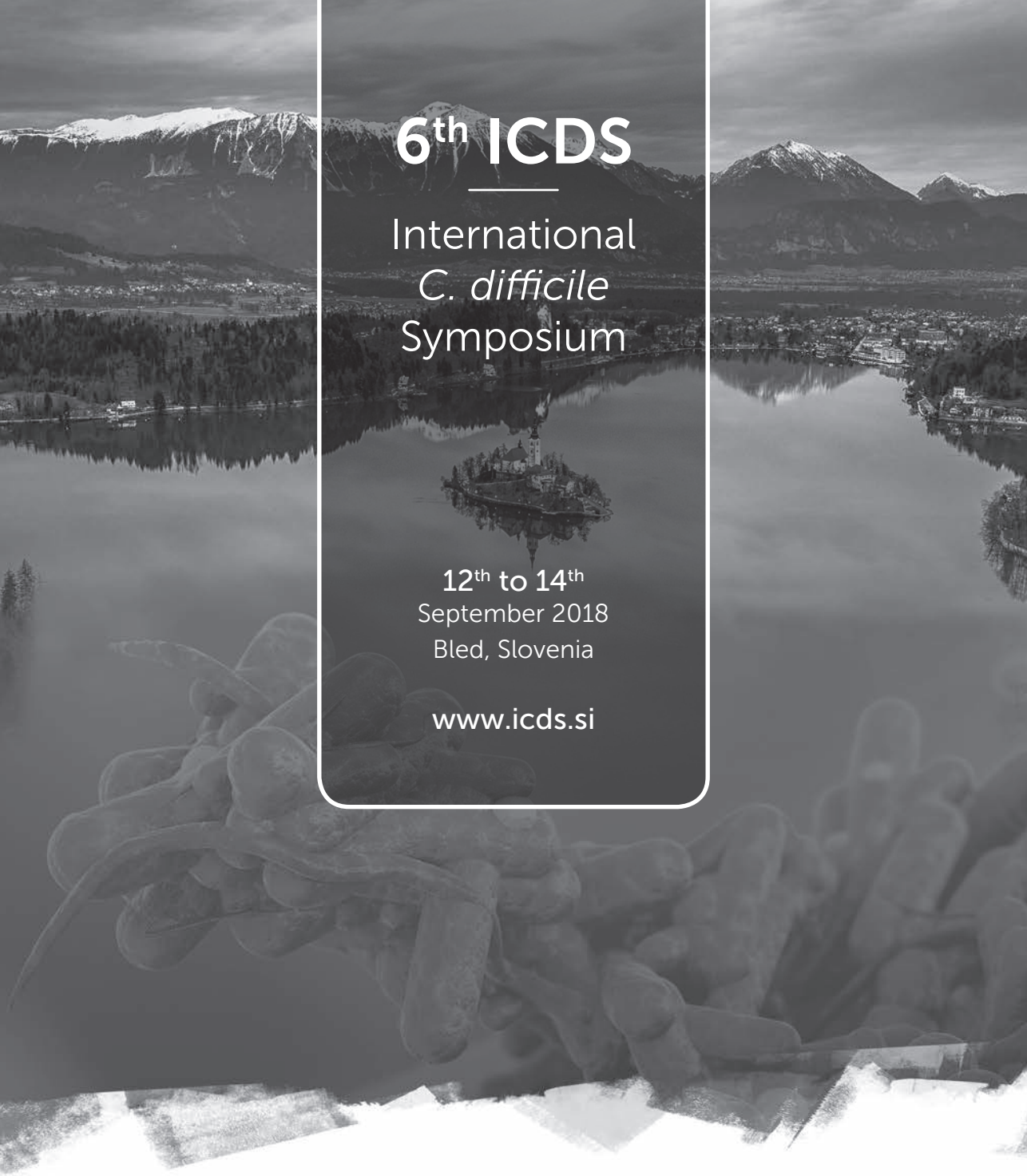
Participant	Country	Institution	Presentation
Levent, Belkis	Turkey	MoH, General Directorate of Public Health	
Lightfoot, Ann	Switzerland	BD Switzerland Sàrl	
Lim, Su Chen	Australia	University of Western Australia	P90, P91
Lindič, Nataša	Slovenia	Jozef Stefan Institute	P37
Logan, Susan	Canada	National Research Council	P47
Louie, Thomas	Canada	University of Calgary	
Maeglin, John	USA	Medscape Education	P76
Magnusson, Cecilia	Sweden	Region Jönköpings län	OP8
Mahnic, Aleksander	Slovenia	National Laboratory for Health, Food and Environment	P13
Maikova, Anna	Russia	Skolkovo Institute of Science and Technology	OP13
Mäkilä, Jenna	Finland	ArcDia International Oy Ltd	
Mannherz, Otto	Germany	R-Biopharm AG	
Marcella, Stephen	USA	Merck Pharmaceuticals	P86
Mathews, Debra	USA	Synthetic Biologics, Inc.	P76
Matos Ferreira, Catia	USA	Pfizer	
Mattila, Eero	Dominican Republic	Helsinki University Hospital	
Maurischat, Sven	Germany	German Federal Institute for Risk Assessment	P96, P97
McBride, Shonna	USA	Emory University School of Medicine	OP17
Meinke, Andreas	Austria	Valneva Austria GmbH	
Mello Viegas, Flávia	Brazil	Federal University of Minas Gerais (UFMG)	
Metzendorf, Nicole	Germany	University of Greifswald	P44
Michel, Annika-Marisa	Germany	TU Braunschweig	P42
Miettinen, Marko	Finland	MSD Finland	
Mihaylova-Mikova, Sashka	Bulgaria	University ""Prof. Dr. Asen Zlatarov""	P72
Mitra, Suparna	United Kingdom	University of Leeds	OP4
Mizrahi, Assaf	France	Groupe Hospitalier Paris Saint Joseph	P2, P3
Moisi, Jennifer	France	Pfizer	
Morgan, Allison	Scotland	MGB Biopharma Limited	
Mortgat, Laure	Belgium	Sciensano	
Moura, Ines	United Kingdom	University of Leeds	P8, P9, P10
Murabata, Mayumi	Japan	Mie University	P83

Participant	Country	Institution	Presentation
Nagy, Elisabeth	Hungary	University of Szeged	
Nale, Janet	United Kingdom	University of Leicester	P48
Nicolas, Isabelle	Belgium	GSK	
Nikolič, Nataša	Serbia	Klinika za infektivne i tropske bolesti KC Srbije	P57
Niwa, Hidekazu	Japan	Equine Research Institute, Japan Racing Association	P92
Noren, Torbjörn	Sweden	Örebro University Hospital	OP8, P78
Normington, Charmaine	United Kingdom	University of Leeds	P8, P9, P10
O'Beirne, Shauna	United Kingdom	University of Sheffield	P32
Oatley, Peter	United Kingdom	University of Sheffield	P31
Oliveira, Pedro	USA	Icahn School of Medicine at Mount Sinai	OP11
Oppenheim, Beryl	France	Cepheid	
Pai, Hyunjoo	Republic of Korea	Hanyang University College of Medicine	P87
Paiva, Ana	The Netherlands	Leiden University Medical center	P34
Péchiné, Séverine	France	Université Paris-Sud	P2, P3
Peltier, Johann	France	Institut Pasteur	P45
Perumalsamy, Sicilia	Australia	University of Western Australia	P99
Piotrowski, Michal	Poland	Medical Uniwersity of Warsaw	P7
Pires, Isadora Honorato	Brazil	Universidade Federal de Minas Gerais	
Poquet, Isabelle	France	INRA National Institute for Agronomic Research	P40
Putsathit, Papanin	Australia	Murdoch University	P84, P99
Rabold, Denise	Germany	Freie Universität Berlin, Germany	P98
Ravic, Miroslav	Scotland	MGB Biopharma Limited	
Regev-Yochay, Gili	Israel	Sheba Medical Center	P88
Rennhack, Kim Eileen	Germany	TU Braunschweig	P35
Riley, Tom	Australia	Pathwest Laboratory Medicine	P18, P19, P50, P84, P86, P90, P91, P99,
Rizzardi, Kristina	Sweden	Public Health agency of Sweden	P78
Rodríguez Díaz, Cristina	Belgium	University of Liège; Faculty of Veterinary Medicine	
Romo, Mariana	Mexico	CONACYT-IMSS	P16

Participant	Country	Institution	Presentation
Roshan Hesari, Niloufar	Australia	University of Western Australia	P50
Rupnik, Maja	Slovenia	National Laboratory for Health, Environment and Food	P13, P101, P102, P103, P105
Rupp, Markus	Germany	MSD Germany	
Salgado, Paula	United Kingdom	ICaMB, Newcastle University	INV10, P20, P24, P27
Sambol, Susan	USA	VA Hines	P55
Savolainen, Roosa	Finland	Vaasa Central Hospital	P65, P69
Schwanbeck, Julian	Germany	Universitätzmedizin Göttingen	P26
Segal, Shoshi	Israel	Sheba Medical Center	
Sekulovic, Ognjen	USA	Tufts University	INV11, OP11, OP14
Sevilla, Eloisa	Spain	University of Zaragoza	INV17, P59, P93
Shaw, Peter	USA	Merck	OP6
Shen, Aimee	USA	Tufts University School of Medicine	OP11
Shivaperumal, Nirajmohan	Australia	University of Western Australia	
Siddiqui, Farida	USA	Hines VA Hospital	P14
Sievers, Susanne	Germany	University of Greifswald	P41, P44
Silva, Rodrigo Otávio	Brazil	Universidade Federal de Minas Gerais	P77
Simons, Andreas	Germany	R-Biopharm AG	
Skok, Pavel	Slovenia	University Medical Centre Maribor	
Smits, Wiep Klaas	The Netherlands	Leiden University Medical Center	INV17, P28, P34, P59
Sorg, Joe	USA	Texas A&amp	INV7
Soutourina, Olga	France	Institute for Integrative Biology of the Cell I2BC	OP13, OP18, P40
Speranza, Nicolas	France	Cepheid	
Starkevic, Urte	Lithuania	Vilnius university	

Participant	Country	Institution	Presentation
Stas, Jeroen	Belgium	GenePOC	P62
Stone, Nathan	USA	Northern Arizona University	OP10
Suarez, Rafael	Switzerland	Ferring International Center SA	
Tamayo, Rita	USA	University of North Carolina at Chapel Hill	INV11, OP11
Tickler, Isabella	USA	Cepheid	P79
Tkalec, Valerija	Slovenia	National Laboratory for Health, Environment and Food	P103
Tosi, Alessandro	Italy	Meridian Bioscience	
Troitzsch, Daniel	Germany	University Greifswald	P41
Tsai, Pei-Jane	Taiwan	National Cheng-Kung University	P4
Turk, Dusan	Slovenia	Jozef Stefan Institute	P37
Usenik, Aleksandra	Slovenia	Jozef Stefan Institute	P37
Van Broeck, Johan	Belgium	National reference center C.difficile (Université catholique de Louvain)	P64
Vazquez, Silvia	Spain	Hospital General Universitario Gregorio Marañón	P49, P75
Vedantam, Gayatri	USA	Unoversity of Arizona	INV2
Vendrik, Karuna	The Netherlands	Leids Universitair Medisch Centrum	OP7,P51
Vernon, Jon	United Kingdom	University of Leeds	P60
von Eichel-Streiber, Christoph	Germany	tgcBIOMICS GmbH	OP3
Vrabic, Tanja	Slovenia	National Laboratory for Health, Environment and Food	P102
Wang, Ziyi	United Kingdom	Newcastle University	P27
Webber, Chris	USA	Pfizer, Inc.	
Whittle, Michaella	United Kingdom	University of Nottingham	OP12
Wilcox, Mark	United Kingdom	University of Leeds / Leeds Teaching Hospitals NHS Trust	INV6, OP4, OP6, P5, P8, P9, P10, P25, P52, P60, P77
Wojtacka, Joanna	Poland	University of Warmia and Mazury in Olsztyn, Faculty of Veterinary Medicine	P95

Participant	Country	Institution	Presentation
Wu, Yuan	China	Chinese Center for Disease Control and Prevention	P58
Zilberman Daniels, Tal	Israel	Sheba Medical Center	


6th ICDS
International
C. difficile
Symposium
12th to 14th
September 2018
Bled, Slovenia
www.icds.si

NOTES

Notes

Notes

Notes

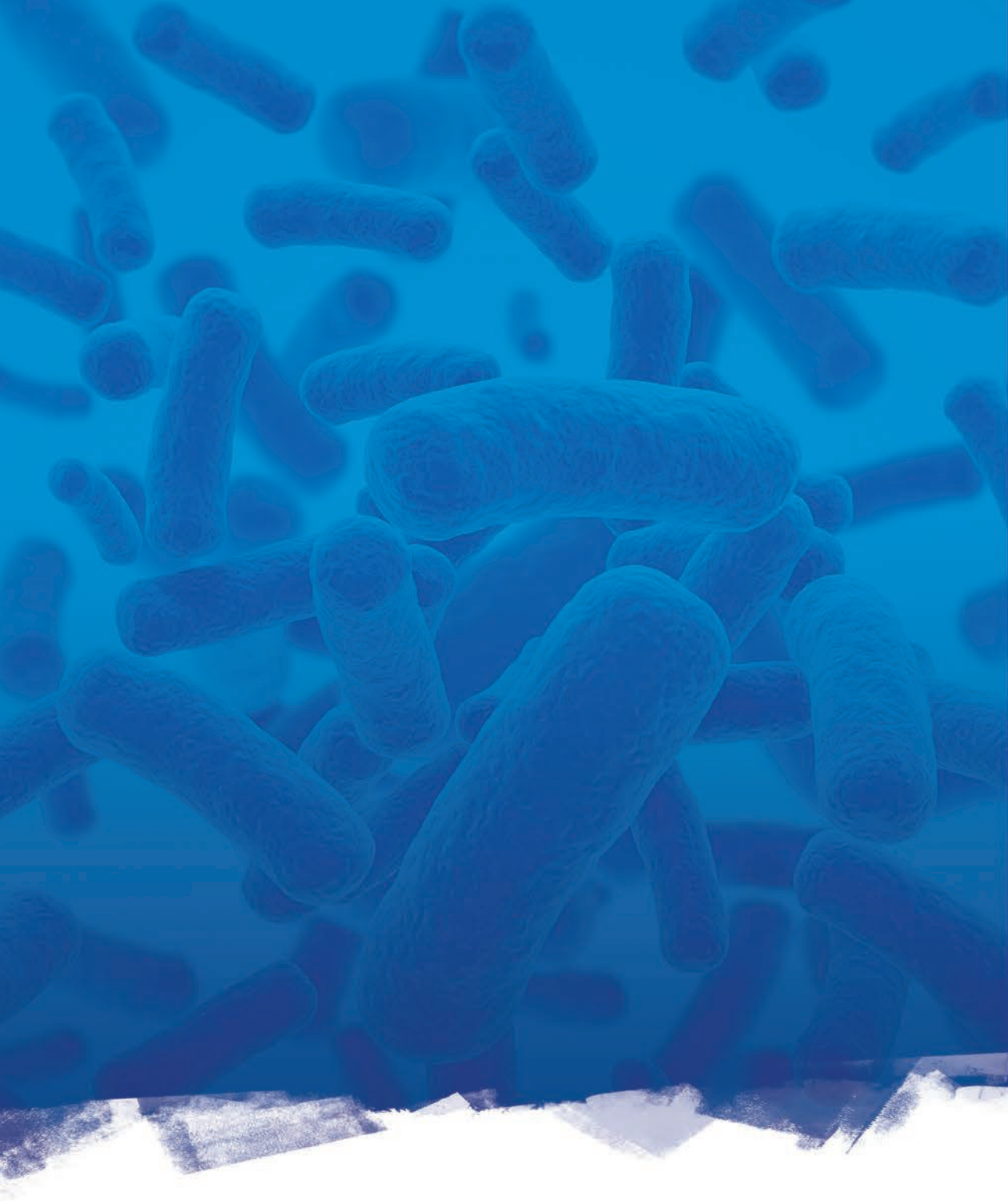
Notes

Notes

Notes

Notes

Notes



NACIONALNI LABORATORIJ ZA
ZDRAVJE, OKOLJE IN HRANO