CLOSTPATH 11

Leiden, August 19-22, 2019

ABSTRACT BOOK

Stadsgehoorzaal, Leiden, The Netherlands

11th International Conference on the Molecular Biology and Pathogenesis of the Clostridia

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Young Scientist Committee members are supported by a FEMS Meeting Grant

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OVERVIEW OF PREVIOUS CLOSTPATH EDITIONS

EDITION		LOCATION	ORGANIZER
Clostpath 1	(1995)	Rio Rico, United States	Glenn Songer
Clostpath 2	(1997)	Seillac, France	Stewart Cole
Clostpath 3	(2000)	Chiba, Japan	Tohru Shimizu
Clostpath 4	(2003)	Woods Hole, United States	Linc Sonenshein
Clostpath 5	(2006)	Nottingham, United Kingdom	Nigel Minton
Clostpath 6	(2009)	Rome, Italy	Paola Mastrantonio
Clostpath 7	(2011)	Ames, United States	Glenn Songer
Clostpath 8	(2013)	Palm Cove, Australia	Julian Rood
Clostpath 9	(2015)	Freiburg, Germany	Klaus Aktories
Clostpath 10	(2017)	Ann Arbor, United States	Vincent Young

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WELCOME TO CLOSTPATH 11.

It is a great pleasure for me to welcome you to the eleventh international CLOSTPATH meeting in Leiden. This international conference brings together clinicians and scientists with various backgrounds and interests. Fundamental research topics form the basis for presentations and discussions on infections by *Clostridium perfringens, C. botulinum, C. tetani, C. sordellii* and *Clostridioides difficile*. Similar as in 2017, we have included topics that cover the host factors that predispose for clostridial infections and also invited companies to participate and update us with (very) recent findings that may be of importance for the scientific community.

Since young scientists are extremely important to develop and test new hypotheses and to bridge basic research with clinical studies, we decided to offer for the first time a **Pre-CLOSTPATH Young Scientists Meeting**, that is organized by and for junior scientists, on the day preceding the main conference at no additional cost. Here, junior scientists have an opportunity to present their work to their direct peers, and get to know them better to enhance their networking experience later on.

It is an honour for Leiden to organize this meeting. Leiden is home to the oldest university of the Netherlands (1575). The “Stadsgehoorzaal Leiden” is an historical location in the city centre of Leiden. This unique building rests on the foundations of the Saint Catharina hospital, which dates back to 1276. The Netherlands has a rich tradition in microbiology, from the first microscope by Anthonie van Leeuwenhoek (on display in Rijksmuseum Boerhaave in Leiden) to the first microbe-museum in the world, Micropia, in nearby Amsterdam. Leiden University Medical Center (LUMC) hosts the National Reference Laboratory for *Clostridioides difficile,* the Centre for Microbiota Analysis and Therapeutics (CMAT) and the National Donor Faeces Bank (NDFB) of the Netherlands. For these and other reasons, CLOSTPATH 11 will also feature sessions on the role of microbiota in clostridial disease.

We hope that CLOSTPATH 11 will be an exciting meeting and are very much looking forward to meet you in Leiden!

On behalf of the organizing committee,

Prof. dr. Ed J. Kuijper

GENERAL INFORMATION

Conference venue
Stadsgehoorzaal Leiden
Breestraat 60
2311 CS Leiden
The Netherlands
Tel: + 31 71 516 3881

Registration
Registration and information desk opening hours

Monday, August 19	15.00 – 17.00 hrs.
Tuesday, August 20	08.00 – 21.30 hrs.
Wednesday, August 21	08.00 – 15.30 hrs.
Thursday, August 22	08.00 – 18.30 hrs.

Badges
With your registration materials, you will receive a personalized name badge. It grants access to the scientific sessions, welcome reception and exhibition. During the conference, this badge must be worn visibly at all times.

Optional Social Activities
There are several activities planned on Wednesday, 21st August from 15:00. If you are registered for one of the tours, you have received the information in regards to the pick-up location and timing via e-mail.
If you would like to join any activity last-minute please check at the registration desk if there are still tickets available.

Certificate of attendance
The certificate of attendance will be provided to you after the Conference via e-mail together with an online survey to evaluate your experience.

Oral Communication
Please hand over your presentation at least one hour or in the break prior to your presentation to the conference AV-support present.

Poster Communication
There are two scheduled poster sessions during the conference:
- **Tuesday August 20 // 15:30 hrs – 18:00 hrs**
  – odd poster numbers
- **Thursday August 22 // 14:50 hrs – 17:15 hrs**
  – even poster numbers

Poster presenters should be at their poster during the their scheduled poster session. The posters should be on display continuously from Tuesday August 20 10:00hrs until Thursday August 22 18.00hrs. Posters should be removed latest on Thursday August 22 at 18:30hrs, immediately after the closing session. Posters that have not been taken away by 19:00hrs will be removed by the Conference Organizers.

Social media
Twitter users can use the hashtag #clostpath2019 to communicate about the meeting. Please respect the indicated preference of presenters when using social media and/or photography and obtain permission when necessary.

Internet access
Complimentary WiFi is available for all participants in the conference venue.
Use the following credentials to log on:

Network: Clostpath2019
Password: clostpath11

Contact
Most information will be available on the conference website:
www.clostpath2019.org.
For additional matters you can contact us during office hours at:
+31 (0)88 089 81 01
For urgent matters onsite at the venue please see us at the registration desk or call our cell: 31 (0) 6 121 56 307.

Meeting Secretariat CLOSTPATH 11
Congress by design
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LIST OF INVITED SPEAKERS

David Aronoff	United States
Martha Clokie	United Kingdom
Craig Ellermeier	United States
Yukako Fujinaga	Japan
Lindsay Hall	United Kingdom
Rongsheng Jin	United States
Hilary Browne	United Kingdom
Mauricio Navarro	United States
Ornella Rossetto	Italy
Olga Soutourina	France
Sarah Tschudin-Sutter	Switzerland
Mark Wilcox	United Kingdom
Vincent Young	United States
Joseph Zackular	United States
Ed Kuijper	The Netherlands

GRANTS AND AWARDS

FEMS ATTENDANCE GRANT

Manuela Fuchs	Germany
Lucy Frost	United Kingdom
Abbie Kelly	United Kingdom
Hannah Fisher	United Kingdom
Sara Ramalhete	Portugal

NIH ATTENDANCE GRANT

Not yet known at time of printing

NDFB ATTENDANCE GRANT

Emilie van Lingen	The Netherlands
Eline Boeije – Koppenol	The Netherlands
Karuna Vendrik	The Netherlands

ESCMID ATTENDANCE GRANT

Matilda Berkell	Belgium
Michal Piotrowski	Poland

ESGHAMI ATTENDANCE GRANT

Raymond Kiu	United Kingdom

ASM POSTER PRIZE

Not yet known at time of printing

PROGRAM OVERVIEW

MONDAY AUGUST 19, 2019		
15:00-17:00	Registration	
17:00-19:00	Welcome reception/mixer at Town Hall **HOSTED BY THE MAYOR OF LEIDEN**	
19.00-19.30	Bites at Stadsgehoorzaal	
19:30-20:30	From microbiome to machine learning to study *C. difficile* infections	**KEY 1**: VINCENT YOUNG CHAIR: ED KUIJPER

TUESDAY AUGUST 20, 2019		
SESSION 1	THEME: GENE REGULATION	CHAIR: DENA LYRAS
08:30-09:00	Regulatory RNAs in *C. difficile*: functions and applications **Tohru Shimizu Memorial Lecture**	**INV 1:** OLGA SOUTOURINA
09:00-09:20	DdlR, an essential transcriptional regulator of peptidoglycan biosynthesis in *C. difficile*	**O 1:** BORIS BELITZKY
09:20-09:40	A novel conserved protein complex regulator controls sporulation and toxin production in *C. perfringens*	**O 2:** NAOKI MUTO
09:40-10:00	Global discovery of Hfq-associated sRNA-target networks in *C. difficile*	**O 3:** MANUELA FUCHS
10:00-10:30	**COFFEE BREAK**	
SESSION 2	THEME: TOXINS	CHAIR: MIIA LINDSTRÖM
10:30-11:00	The role of the single interchains disulfide bond in Tetanus and Botulinum Neurotoxins and the development of anti-tetanus and anti-botulism drugs	**INV 2:** ORNELLA ROSSETTO
11:00-11:20	Using Inhibitors to Probe the Involvement of RIP1, RIP3 and MLKL in Caco-2 Cell Death Caused by *C. perfringens* Enterotoxin	**O 4:** ARCHANA SHRESTA
11:20-11:40	Identification and characterization of the cellular receptor for *C. sordellii* TcsL Lethal Toxin	**O 5:** ROMAN MELNYK
11:40-12:00	The pore structure of *Clostridium perfringens* epsilon toxin	**O 6:** MONIKA BOKORI-BROWN
12:00-12:30	Structural basis of receptor recognition by *C. difficile* toxin B	**INV 3:** RONGSHEN JIN

12:30-13:30	**LUNCH**	
SESSION 3	THEME: CLINICAL/EPIDEMIOLOGY	CHAIR: ERIK DUBBERKE
13:30-14:00	Gut model evidence of effective CDI therapeutics	**INV 4:** MARK WILCOX
14:00-14:20	Building a routine national clinical genomics service for real-time tracking of *C. difficile* to support better infection control	**O 7:** THOMAS CONNOR
14:20-14:40	Botulism – old threat with novel features	**O 8:** MIIA LINDSTRÖM
14:40-15:10	*C. difficile* – challenges for infection control	**INV 5:** SARAH TSCHUDIN-SUTTER
15:10-15:30	**Flash talks**	CHAIR: JEROEN CORVER
15:30-18:00	**COFFEE BREAK AND POSTER SESSION 1**	
18:00-20:00	**DINNER AT STADSGEHOORZAAL**	
20:00-21:00	Clostridia and spore evolution in the gut microbiome	**KEY 2** HILLARY BROWNE CHAIR: ED KUIJPER

WEDNESDAY AUGUST 21, 2019		
SESSION 4	THEME: ONE HEALTH	CHAIR: CÉSAR RODRÍGUEZ
08:30-09:00	*C. perfringens* enteric infections	**INV 6:** LINDSAY HALL
09:00-09:20	*C. perfringens* type C enteritis in pigs: an overview of clinics, pathology, pathogenesis and prevention	**O 9:** HORST POSTHAUS
09:20-09:40	Antibody response of bovines vaccinated with the recombinant Hc domain of botulinum neurotoxin type C and D	**O 10:** LUCA BANO
09:40-10:00	Plasmid-mediated metronidazole resistance in *C. difficile*	**O 11:** ILSE BOEKHOUD
10:00-10:30	Pathobiology of *C. perfringens* type F infections in animal models	**INV 7:** MAURICIO NAVARRO
10:30-11:00	**COFFEE BREAK**	

SESSION 5	THEME: FUNDAMENTAL BIOLOGY	CHAIR: AIMEE SHEN
11:00-11:30	Lysozyme resistance mechanisms	**INV 8:** CRAIG ELLERMEIER
11:30-11:50	Transcriptomic, proteomic and functional analysis of the plasmid pCS1-1 during spore formation in *Clostridium sordellii*	**O 12:** SAMANTHA MUNN
11:50-12:10	The serine proteases CspA and CspC are essential for *C. perfringens* SM101 spore germination through activating SleC and cortex hydrolysis	**O 13:** MAHFUZ SARKER
12:10-12:30	Structure and assembly of S-layer in *C. difficile*	**O 14:** PAULA SALGADO
12:30-13:30	**LUNCH**	
SESSION 6	THEME: IMMUNOLOGY/VACCINES	CHAIR: CLAIRE JANOIR
13:30-14:00	Harnessing eicosanoid biology to prevent CDI	**INV 9:** DAVID ARONOFF
14:00-14:20	Colitis-induced Th17 cells increase the risk for severe subsequent *C. difficile* infection	**O 15:** MAHMOUD SALEH
14:20-14:40	The Role of Dendritic Cells in *C. difficile* Infection	**O 16:** XINGMIN SUN
14:40-15:00	Host immunity modulates efficacy of fecal microbiota transplantation treatment of *C. difficile* infection	**O 17:** MICHAEL ABT
	SOCIAL PROGRAM	
	SELF-ORGANIZED DINNER	

THURSDAY AUGUST 22, 2019		
SESSION 7	THEME: PATHOGENESIS	CHAIR: BORDEN LACY
08:30-09:00	Mechanisms of pathogenesis of botulinum neurotoxin complex	**INV 10:** YUKAKO FUJINAGA
09:00-09:20	Evaluating whether claudin-1 affects the action of *C. perfringens* enterotoxin on Caco-2 cells	**O 18:** IMAN MEHDIZADEH GOHARI
09:20-09:40	The role of siderophores in iron acquisition during *C. difficile* pathogenesis	**O 19:** JESSICA HASTIE
09:40-10:00	The detection and isolation of ε-toxin producing *C. perfringens* from Multiple Sclerosis patients	**O 20:** DAVID SANNINO
10:00-10:20	Differential involvement of S100B/RAGE signaling in C. *difficile* toxin-induced inflammation and apoptosis in enteric glial cells	**O 21:** CIRLE WARREN

10:20-10:50	**COFFEE BREAK**	
SESSION 8	THEME: THERAPEUTICS/ALTERNATIVES	CHAIR: NIGEL MINTON
10:50-11:20	The biology and practical applications of bacteriophages associated with *C. difficile*	**INV 11:** MARTHA CLOKIE
11:20-11:40	Anti-sporulation strategies targeting *C. difficile* and other spore-forming bacterial pathogens	**O 22:** YOGITHA SRIKHANTA
11:40-12:00	Strain Colonization of a Defined Bacterial Consortium and Restoration of the Microbiota and Metabolite Pool of Healthy Volunteers	**O 23:** JASON NORMAN
12:00-12:20	Fidaxomicin association with the exosporium of *C. difficile* spores	**O 24:** EUGÉNIE BASSÈRES
12:20-13:20	**LUNCH**	
SESSION 9	THEME: MICROBIOTA	CHAIR: VINCENT YOUNG
13:20-13:50	Pathogen-microbiota interactions during *C. difficile* infection	**INV 12:** JOSEPH ZACKULAR
13:50-14:10	Divergent host and metabolic responses to fecal microbiota transplantation from different hosts in a mouse model of *C. difficile* infection	**O 25:** ANNA SEEKATZ
14:10-14:30	Microbiota-regulated IL-33 restricts the severity of *C. difficile* infection via Type 2 Innate Lymphoid Cell	**O 26:** WILLIAM PETRI
14:30-14:50	**Flash talks**	CHAIR: JEROEN CORVER
14:50-17:15	**COFFEE BREAK AND POSTER SESSION 2**	
17:15-17:45	From epidemiology to the Netherlands Donor Feces Bank	**Closing lecture:** ED KUIJPER CHAIR: WIEP KLAAS SMITS
17:45-18:00	Summary and announcements	
19.30-.....	**CONFERENCE DINNER AND PARTY AT SCHELTEMA**	

FRIDAY AUGUST 23, 2019	
	Departure

Program may be subject to change.

ABSTRACTS

KEY 1

From the microbiome to machine learning: systems biology of *Clostridioides*

Vincent B. Young[1], William Henry Fitzbutler[1]
[1] *The University of Michigan, Department of Internal Medicine/ Division of Infectious Diseases, Department of Microbiology & Immunology, United States*

The development of *Clostridioides difficile infection (CDI)* represents the outcome of interactions between the bacterial pathogen, the host and the indigenous microbiota of the intestinal tract. CLOSTPATH 11 will feature some of the latest research on all aspects of CDI related to each of these key players. Our group of investigators has been supported by an NIH award that charged us with employing a holistic, systems biology approach to start to decipher the complexity of the interactions between *C. difficile*, the host and the gut microbiota. We have been investigating CDI in hospitalized patients and in murine models of experimental infection with *C. difficile*. Using a multi-omic approach, we have been investigating the emergent properties of the complex biologic system represented by CDI to develop predictive, multiscale models that determine the factors that influence susceptibility to CDI and ultimately the clinical outcome of infection. We have characterized the gut microbiota in terms of structure and function through a combination of culture-independent surveys of the microbiota in patients and experimentally infected mice as well as metagenomic and metabolomic characterization of the intestinal microbiome. Host responses have been delineated during CDI by a combination of serum and fecal biomarkers including cytokines in human and murine CDI and flow cytometric analysis of the cellular response of experimentally infected mice. Further characterization of host factors important in CDI has come through data-intensive, machine learning approaches that leverage the information stored in the electronic health record (EHR). Finally, we have been analyzing genomic and phenotypic features of *C. difficile* strains from patients with CDI that are associated with various clinical outcomes. In this talk I will highlight some of the insights that have arisen through our collaborative, interdisciplinary approach to CDI. This will include a greater understanding of the metabolic environment that the microbiota and host create in the gut that influences the biology of *C. difficile*, the development of EHR-based models for predicting risk of developing CDI in hospitalized patients and predicting clinical outcome of those who become infection, and novel understanding of the epidemiology of CDI in patients that comes from whole genome analysis of clinical strains. These holistic, integrative studies of the emergent features of *C. difficile* infection will hopefully provide a framework to consider the multiscale presentations on the pathogenesis of disease-related *Clostridium* that will fill the next three days of CLOSTPATH 11.

Conflict of interest: Yes
Disclosures: Consultant to Vendanta Biosciences, BioK+ International, Pantheryx, Exarca Pharmaceuticals

KEY 2

Transmission of spore-forming commensal and pathogenic human-associated bacteria

Hilary Browne[1]

[1]*Wellcome Sanger Institute, Hinxton, United Kingdom*

Humans are colonised by diverse microbial communities that provide multiple health benefits. The first major colonisation event is at birth but bacterial transmission and colonisation between individuals continues throughout life. Transmission of intestinal commensal bacteria that promote human health through their metabolic activities and resistance to pathogens is therefore an important but underappreciated factor that functions to maintain the intestinal microbiota. Commensal bacteria use similar transmission routes as pathogenic bacteria, including the ability to make resilient spores, hence, control measures designed to block pathogen transmission may also impede commensal transmission. As such, a greater awareness and understanding of the transmission of human-associated commensal bacteria is required. In this talk I will discuss how transmission between hosts has impacted the evolution and intestinal ecology of human-associated pathogenic and commensal bacteria with a focus on transmission of anaerobic spore-forming bacteria.

Conflict of interest: No

INV 1

Regulatory RNAs in *Clostridium difficile:* functions and applications

Olga Soutourina[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. Clostridia largely use complex RNA-based mechanisms to finely control their gene expression. We are interested in the roles of ncRNAs in the pathophysiology of major human enteropathogen *Clostridium difficile*. We have recently identified a great number (>200) and a large diversity of ncRNAs of different classes in *C. difficile* including abundant CRISPR (clustered regularly interspaced short palindromic repeats) RNAs for defence against foreign DNA, cis-antisense RNAs, riboswitches and trans riboregulators requiring the RNA chaperone protein Hfq. These ncRNAs might play important roles in the control of gene expression during the *C. difficile* infection including metabolic adaptations, biofilm formation, stress responses, defence mechanisms and sporulation. Our goal is to determine the biological roles of selected ncRNAs and to uncover the molecular mechanisms of RNA-based regulations employed by *C. difficile*. Recent advances in elucidation of the original features of RNA-based mechanisms in this important enteropathogen will be discussed. This pathogen must cope with foreign DNA invaders and multiple stress factors inside the host. We have reported an experimental evidence of defensive function of the *C. difficile* CRISPR-Cas system important for its survival within phage-rich gut communities. The use of endogenous CRISPR-Cas system provides an efficient genome editing tools for *C. difficile*. We also described the identification of type I toxin-antitoxin (TA) systems with the first functional antisense RNAs in this pathogen. The characterization of regulatory RNAs has a great potential for future development of new antibacterial strategies based on antisense RNA targeting, as well as TA and CRISPR system applications to limit the pathogen development.

Conflict of interest: No

INV 2

The role of the single interchain disulfide bond in botulinum neurotoxins and the development of anti-botulism drugs

Ornella Rossetto[1], Marco Pirazzini[1], Cesare Montecucco[1]
[1] *Dept. of Biomedical Sciences, University of Padova, Padova, Italy*

A large number of bacterial toxins consist of active and cell binding protomers linked by an inter-chain disulfide bridge. The largest family of such disulfide bridged exotoxins is that of the Clostridial neurotoxins that consist of two chains and comprise tetanus neurotoxins (TeNT) causing tetanus and many botulinum neurotoxins (BoNT) causing botulism. The widespread diffusion of fast and cheap next generation sequencing techniques revealed that neurotoxigenic clostridia have considerable genetic heterogeneity and most importantly, toxin sequence variability. Indeed, many botulinum neurotoxin variants/subtypes have been identified and many others are likely being discovered. Accordingly, a major challenge in the field is identifying inhibitors that would antagonize the neuroparalytic activity of botulinum neurotoxins by acting independently of their immunological properties and/or the structural and biochemical features of the catalytic domains. Recent studies led to the identification of the redox couple Thioredoxin Reductase-Thioredoxin (TrxR-Trx) as the responsible for reduction of the interchain disulfide bond of botulinum neurotoxins and to the very relevant finding that drugs that inhibit TrxR-Trx also prevent botulism. Therefore, molecules, which block the reduction of BoNT interchain disulphide bond can be considered as lead compounds for the development of pan-inhibitors of BoNTs regardless of the toxin variant causing intoxication. In addition, TrxR-Trx specific drugs such as Ebselen and PX-12, previously used in trials in humans, satisfy all the requirements for clinical tests to be performed to evaluate their capacity to effectively counteract human and animal botulism arising from intestinal toxemias such as infant botulism.

Conflict of interest: No

INV 3

Structural studies of *Clostridium difficile* toxin B

Peng Chen[1], Kwok-ho Lam[1], Zheng Liu[1], Baohua Chen[1], Lan Huang[1], Mark Bowen[2], Hanping Feng[3], Tsutomu Matsui[4], Kay Perry[5], **Rongsheng Jin[1]**
[1]University of California, Irvine, Irvine, United States, [2]Stony Brook University, Stony Brook, United States, [3]University of Maryland Baltimore, Baltimore, United States, [4]SLAC National Accelerator Laboratory, Stanford University, Menlo Park, United States, [5]Argonne National Laboratory, Cornell University, Argonne, United States

Clostridium difficile is an opportunistic pathogen, which establishes in the colon when the gut microbiota is disrupted by antibiotics or diseases. *C. difficile* infection (CDI) leads to diarrhea and life-threatening pseudomembranous colitis, which are largely caused by two homologous virulence factors *C. difficile* toxin A (TcdA) and toxin B (TcdB). While the relative roles of these two toxins in the pathogenesis of CDI are not completely understood, recent studies showed that TcdB is likely more virulent than TcdA and more important for inducing the host inflammatory and innate immune responses.
TcdA (~308 kDa) and TcdB (~270 kDa) contain four functional domains: an N-terminal glucosyltransferase domain (GTD), a cysteine protease domain (CPD), a central delivery and receptor-binding domain (Delivery/RBD), and a C-terminal combined repetitive oligopeptides (CROPs) domain. Numerous structures have been reported for fragments of TcdA and TcdB, which have provided tremendous insights into the functions of these toxin domains. However, it remains unknown how individual domains interact within the supertertiary structure of the holotoxin, and how the holotoxin dynamically responds in a precise stepwise manner to the environmental and cellular cues, such as low endosomal pH and inositol hexakisphosphate (InsP 6), which lead to intoxication. Therefore, we have carried out systematic biophysical studies on the structure of TcdB holotoxin using a combination of X-ray crystallography, small-angle X-ray scattering (SAXS), cross-linking mass spectrometry (XL-MS), and single-molecule fluorescence resonance energy transfer (smFRET). These experiments consistently demonstrate that the elongated CROPs domain is dynamic and can sample open and closed conformations in a pH-dependent manner, which may facilitate modulation of TcdB activity in response to environmental and cellular cues during intoxication.
Further, we have determined several crystal structures of TcdB fragments in complex with neutralizing VHHs, the antigen-binding region (VH) of the heavy-chain-only antibodies (also known as nanobodies or single-domain antibodies,

sdAbs). These structures reveal distinct mechanisms of antibody neutralization of TcdB by inhibiting autoprocessing and activation of the toxin, glucosylation of Rho GTPases, or transmembrane delivery of the toxin into the cytosol. These findings provide the structural basis for antibody engineering to improve their antitoxin activities, or developing multidomain antibodies that simultaneously target multiple neutralizing epitopes on the toxins. Taken together, our studies provide novel insights into the structure and function of TcdB holotoxin and also identify its intrinsic vulnerabilities, which could be exploited to develop new therapeutics and vaccines for the treatment of *C. difficile* infection.

Conflict of interest: Yes
Disclosures: A provisional patent application has been filed by The Regents of the University of California on the use of the structural information on TcdB to prevent and/or treat CDI. R.J. is a co-founder of DesignerT Inc. and H.F. is a co-founder of FZata Inc., which had no role in this study.

INV 4

Gut model evidence of effective CDI therapeutics

Mark Wilcox[1]
[1]*University Of Leeds / Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom*

Almost 20 years ago, we developed a novel colonic model that is seeded with pooled human faeces, and so can closely simulate the (desirable and undesirable) effects of antibiotics, including pathogen (carbapenem resistant enterobacteria {CRE}, fluoroquinolone resistant enterobacteria, enterococci and *C. difficile* {CD}) selection and expansion, microbiome deletion, and antibiotic resistance gene/plasmid transfer. Our gut models, using both planktonic and sessile (biofilm) data, allow us to predict which antibiotics have the lowest/highest risk of CDI risk, and which potential CDI therapeutics are likely to be effective in humans. We have 35 original article publications on these predictive models. Current funders including major UK (e.g. NIHR) and US (e.g. CDC, NIH) organisations, for projects on CDI, dysbiosis, antimicrobial resistance and cystic fibrosis.
The gut models, primed with pooled human faeces, simulate in real-time both human pathogen and microbiome behaviour, during colonisation and infection, under antibiotic exposures. The model has successfully predicted success/failure of CDI treatments in several phase III & IV clinical trials, and has demonstrated resistance emergence and transfer, providing information on selection, proliferation and pathogen fitness. Importantly, the models also reduce dependence on animals for antibiotic testing. The models are highly sought after by industry, helping to circumvent traditional bottlenecks between early drug development and clinical studies. They provide data about the effects of novel and existing antimicrobials, in a well-controlled, clinically reflective system. Model outputs have altered national prescribing policy, helped to design clinical trials, and have enhanced regulatory applications.
Throughput in these models is limited due to their complexity and running time. Recent public funding has been obtained to miniaturise and semi-automate the models, which should provide greater capacity and flexibility.

Conflict of interest: Yes
Disclosures: I have received consulting fees from Abbott Laboratories, Actelion, Antabio, AiCuris, Astellas, Astra-Zeneca, Bayer, Biomèrieux, Cambimune, Cerexa, Da Volterra, The European Tissue Symposium, Ferring, The Medicines Company, Menarini, Merck, Meridian, Motif Biosciences, Nabriva, Paratek, Pfizer, Phico Therapeutics, Qiagen, Roche, Seres, Spero, Surface Skins, Sanofi-Pasteur, Seres, Summit, Synthetic Biologics, Valneva and Vaxxilon; lecture fees from Abbott, Alere, Allergan, Astellas, Astra-Zeneca, Merck,

Nabriva, Pfizer, Roche & Seres; and grant support from Abbott, Actelion, Astellas, Biomèrieux, Cubist, Da Volterra, Merck, MicroPharm, Morphochem AG, Motif Biosciences, Nabriva, Paratek, Pfizer, Sanofi-Pasteur, Seres, Summit and The European Tissue Symposium.

INV 5

Clostridium difficile – challenges for infection control

Sarah Tschudin-Sutter[1]
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Clostridium difficile infection (CDI) is the most important infective cause of healthcare-associated diarrhoea in high income countries and one of the most important healthcare-associated pathogens in both Europe and the United States. It is associated with high morbidity and mortality resulting in both societal and financial burden. A significant proportion of this burden is potentially preventable by a combination of targeted infection prevention and control measures and antimicrobial stewardship.
Given the ongoing significance of CDI in Europe, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) study group for *C. difficile* (ESGCD) published an updated guideline in 2018, which provides a current review of the literature on interventions to control CDI in adult populations in acute health care settings and derives specific infection control recommendations. It aims to provide guidance to those responsible for institutional infection control programs, serving as a reference for best medical practice.
The most important aspects of these recommendations, as well as related challenges will be discussed during this presentation.

Conflict of interest: Yes
Disclosures: Member of the Astellas and MSD Advisory Boards for *C. difficile*, of the Pfizer Antiinfectives Advisory Board for and reports grants from the Swiss National Science Foundation NRP 72 (407240_167060), the Gottfried und Julia Bangerter-Rhyner Stiftung, the Fonds zur Förderung von Lehre und Forschung der Freiwilligen Akademischen Gesellschaft Basel, and the Jubiläumsstiftung from Swiss Life.

INV 6

Clostridium perfringens enteric infections: a focus on preterm infants

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Premature birth, defined as infants born <37 weeks gestation, accounts for 1:9 births globally. Preterm infants have an underdeveloped immune system, are often born via C-section, are prescribed numerous antibiotics, and reside in neonatal intensive care units (NICUs); all of which contribute to disrupting the establishment of the early life gut microbiota. Notably, this altered gut microbial ecosystem has been linked to increased risk of serious infections during NICU stay including necrotising enterocolitis (NEC). NEC is a devastating inflammatory gut disease that manifests as tissue necrosis anywhere along the gastrointestinal tract; with a mortality rate of ~40%. Since the 1970s numerous studies (culture and sequencing-based) have linked overgrowth of *Clostridium perfringens* with NEC. Despite the clinical importance of *C. perfringens* in relation to preterm-NEC infection there are limited studies probing the genomic aspects of isolates obtained from this at-risk patient group. Here I will describe our large-scale study investigating *C. perfringens* in preterm infant cohorts; >250 *C. perfringens* strains obtained from screening >400 preterm infants (both diseased and healthy) in 5 UK-based hospitals. Whole genome sequencing and in-depth bioinformatic analyses were carried out on all isolates, which was complemented with in vitro characterisation on a sub-set of isolates. Findings suggest a potentially hypervirulent *C. perfringens* genotype associated with preterm-NEC, with intra- and inter-hospital transmission of persistent and hypervirulent *C. perfringens* strains also identified. In vitro experiments indicated NEC-associated strains possessed enhanced environmental stress responses, and necrotising capacity against human gut epithelium (also linking to immune readouts in NEC infants), linking to presence of certain toxin-associated genes.

Conflict of interest: No

INV 7

Pathobiology of intestinal infections caused by *Clostridium perfringens* type F in animal models

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Production of *Clostridium perfringens* enterotoxin (CPE) and alpha toxin defines *C. perfringens* type F isolates. CPE is a pore-forming toxin responsible for *C. perfringens* type F food poisoning, one of the most common intestinal bacterial infections in the United States. In addition, CPE is involved in up to 15% cases of human non-food-borne intestinal diseases (NFD), including antibiotic-associated diarrhea. While results of experiments using cell cultures are available, they do not always represent the complex environment present in the intestine of infected hosts. Rabbits and mice have been commonly used to study the pathogenesis of *C. perfringens* type F infections, and molecular Koch's postulates have been fulfilled in both species. Intestinal loops in mice emulate constipation, which is presumed to be responsible for some deaths in humans with *C. perfringens* type F infection, caused by the absorption of CPE from the intestine and its action on distant organs (enterotoxemia) and the induction of hyperpotassemia. Direct inoculation of physiologically relevant doses of CPE into these loops in mice induces dose- and time-dependent mucosal damage, caspase-3 activation, and animal death. Even though caspase-3 mediated apoptosis develops in enterocytes, the use of a pan-caspase inhibitor does not protect from CPE-associated cell damage or lethality, suggesting the involvement of other pathways of cell death (e.g. necrosis and/or necroptosis). The intestinal loop model in mice has also allowed exploring the potential therapeutic effects of mepacrine, an acridine-derivative drug that interferes with CPE pore formation and activity, reducing cytotoxicity in vitro. When administered together with CPE, a high dose of mepacrine was able to protect mice from CPE-induced intestinal damage and lethality; this protection did not involve reduction in the formation of pores, but was able to reduce the level of CPE absorption in some mice. Protection from lethality was associated with the reduction of CPE-induced hyperpotassemia. Furthermore, in vitro studies suggested that NanI sialidase, an extracellular enzyme that releases sialic acids from sialyated host macromolecules, may contribute to intestinal colonization of NFD strains. NanI enhances the adherence of *C. perfringens* type F to intestinal cells and promotes bacterial growth using generated sialic acid as an energy source. When clindamycin-pretreated mice were challenged by oral gavage with equivalent numbers of *C. perfringens* type F strain F4969 or its isogenic *nanI* null mutant, significantly lower numbers of the nanI mutant were recovered from all intestinal segments, and the microorganism was completely cleared from the small intestine by day 4. Complementation of the mutant to restore NanI production also restored colonization capability. These in vivo findings identified NanI as a significant contributor to chronic intestinal colonization by NFD strains. The discovery of new therapeutic alternatives against *C. perfringens* type F will be facilitated by the use of these animal models.

Conflict of interest: No

INV 8

Lysozyme resistance in *Clostridioides difficile*

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

Conflict of interest: No

INV 9

Harnessing eicosanoid biology to prevent CDI

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Eicosanoids are bioactive lipid derivatives of the 20-carbon polyunsaturated fatty acid arachidonic acid and include prostaglandins (PGs) and leukotrienes (LTs). Both PGs and LTs have roles in homeostasis and pathophysiology of the mucosal immune system. We have a strong interest in the role played by eicosanoids in susceptibility to, and response to infection caused by, *Clostridioides difficile*. In a mouse model of antibiotic-associated *C. difficile* infection (CDI), we have determined that the common neutrophil chemoattractant, LTB_4, does not appear to play a role in CDI severity, whereas the endogenous PG molecule PGE_2 appears to be important in mediating epithelial and inflammatory responses to CDI. Pharmacological inhibition of PGE_2 synthesis with a nonsteroidal antiiflammatory drug (NSAID) worsened CDI severity in mice and altered the gut microbiome. On the other hand, therapeutic targeting of PGE_2 signaling with the stable analogue misoprostol reduced CDI severity in mice and appeared to hasten recovery of a normal gut microbiome after antibiotic exposure. These studies suggest that endogenous lipids modulate host vulnerability to severe CDI and can be pharmacologically targeted to reduce illness or possibly to prevent infection.

Conflict of interest: Yes
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INV 10

Mechanism of pathogenesis of botulinum neurotoxin complex and its application

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Food-borne botulism is caused by the ingestion of food contaminated with botulinum neurotoxin (BoNT). To cause illness, BoNT in the gastrointestinal (GI) lumen must traverse the intestinal epithelial barrier. Subsequently, BoNT disseminates through the systemic circulation to target peripheral nerves. BoNTs are produced along with one or several non-toxic protein(s); together, these proteins form progenitor toxin complexes (PTCs) that act as highly potent oral poisons. BoNT/A1 forms two distinct PTCs: hemagglutinin (HA)-negative PTC [HA(–)PTC, M-PTC] and HA-positive PTC [HA(+)PTC, L-PTC and LL-PTC]. HA(+) PTC is the more potent oral toxin. We found that type A1 HA(+)PTC breaches the intestinal epithelial barrier from M cells (intestinal microfold cells) via HA–M cell interaction in order to exert its toxic effects (Matsumura et al., Nature commun. 6: 6255, 2015). M cells are specialized epithelial cells that actively transport luminal macromolecules into the lymphoid follicles and initiate mucosal immunity responses. So, we attempted to use HA as a vehicle for mucosal vaccine. Influenza HA split vaccine was used as an antigen, and nasally administered with or without botulinum HA four times to BALB/c mice. As a result, botulinum HA induced a significant amount of antigen-specific IgA in nasal cavity lavage and bronchoalveolar lavage, whereas antigen alone did not. Another application is the use of HA for the large-scale culture of human induced pluripotent stem cells (hiPSCs). We found that the E-cadherin-specific-inhibiting activity of HA enables highly efficient culturing of hiPSCs in an undifferentiated state. Results of these studies will be described in this presentation.

Conflict of interest: No

INV 11

The biology and practical applications of bacteriophages associated with *C. difficile*

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Clostridium difficile is the most common causal agent of bacterial induced infectious diarrhea in the industrialized world. It causes a significant health and financial burden and is difficult to treat as it is only sensitive to three antibiotics. Bacteriophages, or phages are viruses that specifically infect and kill bacteria, and *C. difficile* phages could play a valuable in treating patients infected with this pathogen. We have isolated a large set of *C. difficile* phages with the view to developing them for next generation therapeutics. The most effective phages have been patent protected and shown to target nearly 90% of clinically prevalent and severe strains that circulate in Europe and the United States.
Exploitation of phages clearly benefits from a thorough understanding of their biology and much of the research in my laboratory has focused on unraveling this. I will show how we used bioinformatic data, protein overexpression and antibody studies to identify their tail fibers and used protein crystallography to identify that it is a novel protein structure. For therapeutic development it is useful to determine the rates of bacterial resistance to phages compared to the rates of resistance towards antibiotics, and to establish how effective they are at killing *C. difficile* in in vitro, ex-situ and in animal models and I will present data on these aspects.
Furthermore, in order to better understand how phages interact with *C. difficile* in a gut setting we have optimized ex-situ models to study *C. difficile*-phage interactions on epithelial cells. We have also optimized biofilm, *Galleria mellonella* (insect), and artificial gut models. Data will be presented on how these approaches can inform us of application regimens, dosage and efficacy and of cellular and microbiome responses following cell lysis. Finally, as all phages that infect *C. difficile* encode integrases, I will present data on engineering these phages, and show how the properties of modified phages compare to the wild type parent phages.

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Conflict of interest: No

INV 12

Pathogen-microbiota interactions during *Clostridium difficile* Infection

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Clostridium difficile is the most commonly reported nosocomial pathogen in the United States and an urgent public health concern worldwide. The primary risk factor for *C. difficile* infection (CDI) is antibiotic use, which reduces colonization resistance to *C. difficile* by perturbing the resident gut microbiota. Despite the well-established link between the gut microbiota and susceptibility to *C. difficile*, little work has been done to explore the impact of *C. difficile*-microbiota interactions during infection. Polymicrobial interactions can be attributed to the development of numerous infections and cross-talk between bacteria is associated with exacerbation of various diseases. One of the most highly abundant members of the microbiota in the *C. difficile*-infected gut is *Enterococcus*. Pathogenic *Enterococcus* share numerous risk factors with *C. difficile* and colonization with vancomycin-resistant *Enterococcus* is associated with increased severity of CDI. We use co-culturing methods, RNA sequencing, and advanced imaging mass spectrometry to explore the effect of the Enterococci on CDI and investigate the molecular mechanisms of cross-talk between *Enterococcus* and *C. difficile*.

Conflict of interest: No

INV 13

From *Clostridioides difficile* epidemiology to the Netherlands Donor Faeces Bank

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In 2005, the hypervirulent *Clostridioides difficile* ribotype (RT) 027 caused multiple outbreaks of *C. difficile* infections (CDI) worldwide. This led to the foundation of the National *C. difficile* Reference Laboratory in the Netherlands and a sentinel surveillance program to register all CDI cases in 22 hospitals in the Netherlands. A mean incidence rate of 2.90 CDI cases per 10,000 patient-days was found and a rapid decline of RT 027 occurred. In 2008, a new hypervirulent RT 078 was detected that is still the third most frequently found type and is also present in many pig farms in the Netherlands. Extrapolating the data of sentinel surveillance to all hospitals in the Netherlands in 2019, it is estimated that approximately 2,710 hospitalized patients will develop CDI, and 85 patients succumb contributable to CDI annually.
Antimicrobial susceptibility tests are regularly performed and resistance to vancomycin, metronidazole and fidaxomycin was not detected until 2017. In December 2017, a first clinical *C. difficile* isolate RT 020 was found in a patient who failed to metronidazole treatment (MIC=8 mg/L). A second treatment failure due to a metronidazole resistant RT 020 isolate was found in 2018.
In the Netherlands, of 2,710 hospitalized patients with CDI, approximately 20% suffer from a relapse and 100 patients develop multiple relapses. Treatment of multiple relapsing CDI (rCDI) is difficult. Since 2016, Fecal Microbiota Transplantation (FMT) has been implemented as an approved treatment approach for rCDI. The Netherlands Donor Feces Bank (NDFB) was founded to facilitate this treatment by providing ready-to-use donor feces suspension. A working group of medical specialists assesses all the FMT requests, to ensure that the indication is correct. Since the start of NDFB in March 2016, 167 FMT requests to treat CDI patients were reviewed within the NDFB working group. Patients with rCDI were treated with a cure rate of 90.4%. Of all the requests, 20.4% of the patients were deemed not suitable for FMT by the NDFB working group.
The linking pin between intestinal colonization with *C. difficile*, development of CDI and application of FMT for multiple rCDI is the role of the microbiota. We performed a case-control study in two large Dutch hospitals and studied 1,527

faecal samples collected from patients at admission for *C. difficile* colonization. Gut microbiota composition from patients with *C. difficile* colonization, without *C. difficile* colonization and patients with CDI were investigated through 16S rRNA gene amplicon sequencing. Surprisingly, *C. difficile* colonized patients were characterised by disturbed microbiota, independent of prior antibiotic use, suggesting that the presence of C. difificile is not indicative for a normal healthy microbiota. A few bacterial species were found to be associated with protection, colonization or development of disease and mechanistic studies are now underway to establish their role.

Conflict of interest: Yes
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O 1

DdlR, an essential transcriptional regulator of peptidoglycan biosynthesis in *Clostridioides difficile*

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D-Ala-D-Ala ligase, encoded by the ddl gene(s), is responsible for the synthesis of a dipeptide, D-Ala-D-Ala, an essential precursor of a pentapeptide stem of bacterial peptidogycan. In *Clostridioides difficile,* the ddl gene is located upstream of the ddlR gene, which encodes a putative transcriptional regulator. Using mutational and transcriptional analysis, gel-shift assays, and DNase I footprinting, DdlR was found to be a direct activator of the ddl ddlR operon in *C. difficile*. DdlR is a member of the poorly characterized but widespread group of MocR/GabR-type bacterial proteins that have DNA-binding and aminotransferase-like, pyridoxal 5'-phosphate-binding domains. Genes, encoding proteins highly similar to DdlR, are present only in classes Clostridia and Bacilli. Though uncommon for transcription regulators, *C. difficile* DdlR is essential, as the *ddlR* null mutant cells could not grow even in compex laboratory media in the absence of D-Ala-D-Ala. D-Ala-D-Ala acts as an anti-activator of DdlR, reducing dipeptide biosynthesis when D-Ala-D-Ala is in excess. The *ddlR* gene is transcribed from the *ddl* promoter but also from its own promoter. A DdlR mutation that prevents covalent binding of PLP abolishes the ability of DdlR to activate transcription. A dyad-symmetry sequence, which is located immediately upstream of the -35 region of the ddl promoter, serves as an important element of the DdlR-binding site. This sequence is conserved upstream of putative DdlR targets in other bacteria, indicating a similar mode of regulation of these genes.

Conflict of interest: No

O 2

A novel conserved protein complex regulator controls sporulation and toxin production in *Clostridium perfringens*.

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Pathogenic bacteria of *Clostridium* form spores to acquire high tolerance to heat, oxygen and antibiotics, causing infectious diseases such as food poisoning. *Clostridium perfringens* is causative bacteria of food poisoning and gas gangrene, and it is considered that sporulation is essential for the pathogenicity of this bacteria. However, the detailed regulatory mechanisms of sporulation by *C. perfringens* are poor understood. In this study, we identified a novel regulator of sporulation and toxin production.
We have shown that disruption of *ctrAB operon* affect the gene expression of various toxins in *C. perfringens* (1). The CtrA and CtrB proteins form stable heteromeric complex in cells, which is indispensable for the regulation of the gene expression. Since the ctrAB operon is highly conserved in several *Clostridium*, we hypothesized that the CtrAB protein complex have an common important role in Clostridia.
We reveal that *ctrB-disrupted* mutant of *C. perfringens* Type F strain SM101 is unable to sporulate, indicating that CtrAB protein complex is essential for sporulation. Microscopic observation of cell morphology reveals that the sporulation in the *ctrB* disrupted strain is arrested at the early sporulation stage (stage 0). In addition, in the *ctrB disrupted* strain, the production of the enterotoxin (CPE) was dramatically reduced. Transcription of the cpe gene requires sporulation-specific sigma factors σE and σK that are required for the progression of the early stages of sporulation in *C. perfringens*. There findings suggest that CtrAB controls the early stages of sporulation.
Spo0A is known to be a primary regulatory protein of sporulation in Bacillus and *Clostridium*. Transcriptome analysis of *ctrAB* and *spo0A* mutant strains shows that more than half of the approximately 200 regulons of *ctrAB* overlaps with that of *spo0A*, imply CtrA-mediated regulation of sporulation is involved in Spo0A. Since CtrAB does not drastically affect the expression of Spo0A, we hypothesized that CtrAB controls the activity of Spo0A. We show that the amount of phosphorylated

Spo0A, which is required for sporulation, is reduced in the *ctrB-disrupted* strain. Our data demonstrate that conserved CtrAB protein complex is a key regulator of initiation of sporulation in *Clostridium*.

Conflict of interest: No

O 3

Global discovery of Hfq-associated sRNA-target networks in *Clostridioides difficile*

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Background
Clostridioides difficile (*C. difficile*) constitutes the leading cause of nosocomial diarrhoea and colitis associated with antibiotic treatment. Although numerous studies have significantly expanded our knowledge of *C. difficile* infection mechanisms, small regulatory RNA-mediated (sRNA-mediated) regulation of virulence and colonization factors remains unexplored.
In Gram-negative species the function of many sRNAs is mediated by the RNA chaperone Hfq, facilitating the base-pairing between sRNAs and their target mRNAs. These interactions may impact mRNA translation and/or stability thereby influencing bacterial physiology and virulence. Despite its crucial role in many Gram-negative bacteria, the function of Hfq in Gram-positive bacteria remains less defined. Initial data indicating a role of Hfq-mediated regulation by sRNAs of several infection-associated pathways has been published (1) but a global identification of Hfq-associated RNAs and their impact on *C. difficile* virulence is still pending.
We aim to characterize the Hfq-mediated sRNA interactome on a transcriptome-wide scale using state-of-the-art RNA-sequencing based approaches: CLIP-seq (cross-linking immunoprecipitation and sequencing) employs in vivo UV cross-linking of Hfq-RNA complexes followed by RNA-sequencing which will provide insight into Hfq target binding sites at single nucleotide resolution thereby revealing structural preferences for Hfq binding to its target RNAs. The RIL-seq (RNA interaction by ligation and sequencing) approach includes an additional ligation step of Hfq-bound RNA-pairs and thus enables in vivo mapping of sRNA-target interactions. Interacting RNA pairs can be identified as chimeric reads that map to two distant genomic regions. Applying these techniques to *C. difficile* grown in different conditions will reveal the dynamic Hfq-regulated sRNA-mRNA networks and their rewiring in response to environmental conditions.
Profiling of Hfq-bound RNAs over a range of growth phases using co-IP of FLAG-tagged Hfq revealed a dynamic spectrum of bound RNAs. Along the time axis of growth, covering mid-exponential, late-exponential and stationary growth phases,

we identified 52 sRNAs and 596 mRNAs (either coding region and/or 5' UTR) that were enriched (log2 ≥ 2) either in a subset or in all three conditions analysed. Hfq-bound non-coding RNAs comprised all known classes of sRNAs including intergenic, antisense and 3' UTR-derived as well as riboswitches. Remarkably, almost all described type-I antitoxins as well as six additional putative type-I antitoxins were enriched, indicating a potential role of Hfq in their regulation. Among the enriched mRNAs we identified a large number of genes involved in metabolic pathways, including the biosynthesis of amino acids, as well as genes involved in translation, membrane transport, cell motility and signal transduction. Ongoing analyses will reveal specific Hfq binding sites (CLIP-seq) as well as sRNA–target interactions (RIL-seq). Together, these approaches will provide the first single nucleotide resolution map of Hfq-target binding sites and reveal the dynamics of the Hfq-mediated post-transcriptional network in response to changing environmental conditions in *C. difficile*.

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Conflict of interest: No

O 4

Using Inhibitors to Probe the Involvement of RIP1, RIP 3 and MLKL in Caco-2 Cell Death Caused by *Clostridium perfringens* Enterotoxin

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Clostridium perfringens type F strains cause a very common food poisoning and many cases of nonfoodborne human gastrointestinal diseases such as antibiotic-associated diarrhea. The enteric virulence of type F strains requires production of *C. perfringens* enterotoxin (CPE) which is a pore-forming toxin. In Caco-2 cells, CPE causes apoptosis at low concentrations but necrosis at high concentrations. Since some forms of necrosis or apoptosis involve RIP1 kinase, RIP 3 kinase, and MLKL pseudokinase, the current study examined the involvement of these three host proteins in CPE-induced apoptosis or necrosis. Biochemical (i.e., lactate dehydrogenase release and caspase-3 activation) assays, microscopy cell death assays, and MLKL oligomerization assays were used to evaluate whether highly specific inhibitors for RIP1, RIP 3, or MLKL reduce CPE-induced apoptosis or necrosis. Results indicated that both RIP1 and RIP 3 contribute significantly to CPE-induced apoptosis and necrosis in Caco-2 cells. In addition, MLKL contributes to CPE-induced necrosis, but not apoptosis. Collectively, these results indicate that the cell death mechanism occurring with high CPE dose-induced cell death is the form of necrosis known as necroptosis. The involvement of RIP1, RIP 3 and MLKL in CPE-induced cell death is likely due to ion dysregulation since previous studies showed, i) cytoplasmic Ca^{2+} and K^{+} changes caused by other toxins can trigger cell death involving these proteins and ii) CPE affects intracellular Ca^{2+} levels. Last, demonstrating the involvement of RIP1, RIP 3 and MLKL in CPE-induced cell death suggests that these three proteins are potential therapeutic targets to protect against CPE action in vivo, particularly since highly-specific inhibitors of RIP1, RIP 3 and MLKL are available.

Conflict of interest: No

O 5

Identification and characterization of the cellular receptor for *Clostridium sordellii* TcsL Lethal Toxin

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Clostridium sordellii lethal toxin (TcsL) is the primary virulence determinant responsible for diseases associated with *C. sordellii* infection, including sepsis, and toxic shock syndrome (TSS). Despite its importance in disease pathogenesis, very little is known about the binding or uptake of TcsL into target cells. Here, we carried out a CRISPR-Cas9-mediated genome-wide knockout screen and identified two members of the widely-expressed semaphorin family as the cellular receptors of TcsL. Loss of sema receptors renders cells completely resistant to TcsL-induced toxicity; while reintroducing sema back into resistant cells through overexpression restores sensitivity to TcsL. Using a series of biophysical approaches we show direct, high-affinity binding between TcsL and the semaphorin ectodomains and show further that recombinant ectodomains can be used as 'decoy' inhibitors of TcsL-mediated cellular toxicity. Through structural characterization and site directed mutagenesis, we localized the binding site to an analogous site as to where Frizzled proteins interact with the homologous toxin TcdB from *C. difficile*. Interestingly, we show however that there is no cross-reactivity between TcsL and frizzled proteins or TcdB and sema proteins, despite the high homology of these toxins. These findings establish the cellular receptor for TcsL and reveal unexpected insights into the similarities and differences between closely related members of the Large Clostridial Toxin family.

Conflict of interest: No

O 6

The pore structure of *Clostridium perfringens* epsilon toxin

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Epsilon toxin (Etx), a potent pore forming toxin (PFT) produced by *Clostridium perfringens*, is responsible for the pathogenesis of enterotoxaemia of ruminants and has been suggested to play a role in multiple sclerosis in humans. Etx is a member of the aerolysin family of β-PFTs (aβ-PFTs). While the Etx soluble monomer structure was solved in 2004, Etx pore structure has remained elusive due to the difficulty of isolating the pore complex. Here we show the cryo-electron microscopy structure of Etx pore assembled on the membrane of susceptible cells. The pore structure explains important mutant phenotypes and suggests that the double β-barrel, a common feature of the aβ-PFTs, may be an important structural element in driving efficient pore formation. These insights provide the framework for the development of novel therapeutics to prevent human and animal infections, and are relevant for nano-biotechnology applications.

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Conflict of interest: No

O 7

Building a routine national clinical genomics service for real-time tracking of *C. difficile* to support better infection control

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In 2018 Public Health Wales NHS Trust (PHW) began collecting and sequencing all Welsh *Clostridioides difficile* samples identified by the UK Anaerobe Reference Unit, Cardiff, in order to develop a routine sequencing service for *C. difficile*. In January 2019, following the completion of bioinformatics and laboratory benchmarking, full real-time, parallel processing using whole genome sequencing began for all *C. difficile* samples reported to PHW by hospitals across Wales.
This presentation will provide an overview of the development of this new service, along with some of the early results that have already been used as part of efforts to track and control *C. difficile* infection in hospitals in Wales.
We built a bioinformatics pipeline to go from reads to reports for *C. difficile* samples. To do this, we first undertook benchmarking comparing the variant (Single Nucleotide Polymorphism) calls generated by the tools Snippy and PHEnix on a previously sequenced collection of 339 *C. difficile* isolates. The initial analysis identified issues with both the selection of an arbitrary reference for variant calling which led to the development of an automated system for reference selection, which was then combined into a pipeline to generate a SNP address for isolates, using SnapperDB.
The complete PHW pipeline, drawing together elements of software developed by Public Health England for use on Salmonella, and custom software built within Public Health Wales is freely available in a variety of reproducible formats on github.
Benchmarking determined that the PHEnix pipeline produced variant calls that were almost completely consistent (>99%) with previous work, such that they could be used in concert with a SNP addressing system to provide a clinical

service for clustering *C. difficile* cases and for characterising the phylogenetic relationships of isolates and clusters of interest.
Our system is scalable, and has already been used to analyse over 1500 clinical samples (covering both retrospective and real-time collection). Each sample is matched to the closest reference (selected from 10 possible *C. difficile* genomes) based on Jaccard distance. Following reference selection, SNP calling and addressing takes place. Cluster data is then integrated and visualised with other epidemiological information via Tableau. Our Tableau interface enables a visual, real-time examination of patient-level information to enable outbreak investigation, which now forms part of *C. difficile* surveillance efforts across multiple hospitals in North Wales.
This work has already enabled the identification of transmission clusters within, and between, hospitals and provides a key, higher-resolution, tool for improved surveillance and control efforts. Within North Wales, our system already forms part of look-backs focused on understanding how *C. difficile* infections could have been prevented.
The emerging national, genomics-based, routine clinical *C. difficile* service within PHW provides an end-to-end solution that could either form the basis of a wider service or act as a template service development elsewhere.
This work demonstrates the potential of genomics for understanding and responding to *C. difficile* infection on a national scale, and provides a platform for an extended ONE-health approach for tracking *C. difficile* in Wales.

Conflict of interest: No

O 8

Botulism – emerging aspects of the old threat

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Botulism risk related to processed and anaerobically stored foods is known for centuries. Botulism is a result of the botulinum neurotoxin (BoNT) blocking neurotransmission and causing a flaccid paralysis that may lead to death upon respiratory muscle collapse. BoNT was first extracted from blood sausages and described by Justinus Kerner in the early 19th century, and a BoNT-producing bacterium, first called *Bacillus botulinus* and later renamed as *Clostridium botulinum,* was isolated almost a century later by the Belgian Emile Pierre-Marie Van Ermengem from a contaminated ham. Research since those days has identified an ever-growing selection of bacterial species able to produce BoNTs, a vast diversity of BoNTs with distinct target species and toxic properties, and several disease forms of botulism with distinct aetiology and epidemiology.
While the classical food poisoning botulism with dramatic symptoms remains as the most common form of botulism worldwide, the increasing number of cases with toxicoinfectious botulism, mild intoxications, and atypical symptomologies raise the question if the epidemiology of botulism is yet fully understood. Recent findings of novel BoNT types and non-clostridial species with BoNT genes, in parallel with population aging, growth of risk groups, dietary changes, and sustainable solutions in food production, are prone to create novel niches for growth and toxin production by BoNT-producing bacteria. This may introduce emerging public health risks. The presentation discusses emerging aspects of botulism.

Conflict of interest: No

O 9

Clostridium perfringens type C enteritis in pigs: an overview of clinics, pathology, pathogenesis and prevention

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C. perfringens type C isolates produce the two major toxins, α (CPA) and β (CPB) and cause severe, acute, necrotizing enteritis in livestock and humans. Outbreaks of human type C enteritis were recorded after the second world war in Germany, but to date, this disease has been reported only sporadically in developed countries. A similar disease was diagnosed more frequently in parts of South East Asia, particularly in the highlands of Papua New Guinea, where it was a cause of childhood mortality until vaccination programmes were initiated. *C. perfringens* type C more frequently causes enteritis in animals such as calves, sheep, goats, and particularly neonatal pigs. This disease in pigs occurs worldwide and can lead to mortality rates of up to 100% in susceptible herds. Recent research has extended our knowledge about the pathogenesis and the prevention of the disease, although important aspects of it remain to be determined. The disease can vary between peracute, acute and subacute to chronic forms. NE is diagnosed in diagnostic laboratories through a combination of pathological and bacteriological investigations. At necropsy, the hallmark lesion of NE is a deep, segmental mucosal necrosis accompanied by massive haemorrhage of the small intestine. C. perfrigens type C can readily be isolated from intestinal samples in acute cases but in subacute to chronic cases it is more challenging to demonstrate pathogenic strains. The pathogenesis of the disease involves an initial rapid overgrowth of *C. perfringens* type C in the small intestine, inhibition of the degradation of CPB by trypsin inhibitors in the colostrum of sows and most likely an initital damage to the epithelial barrier of the small intestine. CPB itself acts primarily on vascular endothelial cells in the mucosa and can also inhibit platelet function. This could explain the acute and prolonged haemorrhage followed by necrosis of the intestinal mucosa. This in turn favours proliferation of the pathogen and upregulation of toxin production, leading to a vicious cycle ending in rapidly progressing tissue necrosis. Once affected, the disease progresses so rapidly that animals usually die within hours of few days. Prevention of the disease is achieved by immunization of pregnant sows with *C. perfringens* type C toxoid vaccines, combined with proper sanitation on farms. After a basic immunization during the first pregnancy, sows have to receive booster vaccinations during every subsequent pregnancy to achieve sufficient passive immunity in piglets. Nevertheless, a proportion of piglets, especially from primiparous sows, seems to be insufficiently protected from NE. Simple adaptations of the immunization scheme however can improve herd immunity and decrease the risk of outbreaks of NE. Currently it is however unknown how exactly vaccinations confers protection against disease.

Conflict of interest: No

O 10

Antibody response of bovines vaccinated with the recombinant Hc domain of botulinum neurotoxin type C and D

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In the present study, we compared the immune response of bovines immunized with the recombinant heavy chain (Hc) domain of botulinum neurotoxin (BoNT) type C and D, with the immune response elicited by a commercial toxoid based vaccine.

The C-terminal part of the Hc of botulinum neurotoxin types C and D (HcBoNT/C and D) was expressed in *Escherichia coli* and purified as previously described. Ten bovines were vaccinated twice with a bivalent (C and D) traditional toxoid-based vaccine, 10 bovines three times with the experimental recombinant vaccine, 5 bovines were injected three times with the adjuvant and 5 cohort bovines were included in the study as negative control. Serum samples were collected before the first vaccination and three weeks after the second and the third vaccination. To evidence the antibody response, two ELISA tests were developed in-house using the immunogens as capture antigens. The neutralising antibodies were investigated by means of a mouse protection assay (MPA) performed with BoNT type C and D/C. The neutralizing titre was quantify by using two reference antitoxins.

The group of bovines vaccinated with the recombinant vaccine showed an average antibody titre of 1.031 ELISA units (EU) for type C and 1.14 EU for type D. The average antibody titre of control animals and animals inoculated with the only adjuvant was 0.171 EU and 0.097 EU respectively, when tested with HcBoNT/C as capture antigen, and 0.013 EU and 0.007 EU when tested with the HcBoNT/D. The average antibody titre of bovines vaccinated with the bivalent toxoid vaccine was 0.461 EU for type C and 0.182 for type D. After two vaccinations, the mouse protection assay performed with BoNT type C revealed an average neutralizing titre of 4 to 8 International Units (UI)/ml in bovines vaccinated with the recombinant vaccine and an average titre of 0.25 to 0.5 UI/ml in bovines vaccinated with the toxoid vaccine. Despite the high humoral response evidenced by ELISA, the mouse

protection assay performed with BoNT type D/C did not show any protection titre after two vaccinations and the antibody response recorded after the third vaccination was equal (1.25-2.5 UI/ml) to that obtained with the toxoid vaccine after two vaccinations. This result is probably due to the limited homology of the HcBoNT/DC with the HcBoNT/D (37%) and HcBoNT/C (77%) (1).
Our results demonstrate that two vaccination with recombinant HcBoNT/C are able to elicit a titre of neutralizing antibodies at least 8 times higher than a conventional toxoid vaccine. To prevent bovine botulism sustained by BoNT/DC, a new subunit vaccine set up with the C-terminal part of the heavy chain of the mosaic BoNT/DC should be tested. The investigation on the capability of HcBoNT/D to elicit protective antibodies against non-mosaic BoNT/D, is in process.

1. Nakamura K, Kohda T, Shibata Y, Tsukamoto K, Arimitsu H, Hayashi M, Mukamoto M, Sasakawa N, Kozaki S. 2012. Unique biological activity of botulinum D/C mosaic neurotoxin in murine species. Infect Immun 80:2886-2893.

Conflict of interest: No

O 11

Metronidazole resistance in *Clostridium difficile* is conferred by a plasmid

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Although the consensus is shifting towards vancomycin, current European treatment guidelines indicate the use of metronidazole for mild- to moderate *Clostridium difficile* infections (CDI) [1]. Several mechanisms for metronidazole resistance have been described, but none of them are plasmid-mediated in *C. difficile*. A patient treated in the Leiden University Medical Center (LUMC) suffered from recurrent CDI caused by a RT020 strain that was initially susceptible to metronidazole (MTZS), but later during the infection became metronidazole resistant (MTZR, MIC ≥ 8 g/L). Using whole genome sequencing (WGS) analysis we analyzed these MTZS and MTZR patient isolates and included both MTZS- and MTZR RT010 isolates from the collection of the national reference laboratory of the LUMC/RIVM. We found a plasmid, pCD-METRO, which correlated with metronidazole resistance.
A total of 18 strains (6 MTZS, 12 MTZR) were analyzed with core genome SNP analysis. A large collection of clinical isolates from human- and animal origin was tested for the presence of pCD-METRO by PCR. By inserting a shuttle module into pCD-METRO, the plasmid was conjugated into the MTZS lab strain 630Δerm. Resistance to metronidazole was measured by E-test, and agar dilution according to CLSI standards.
WGS data showed that the RT020 strains (MTZS and MTZR) were clonal. pCD-METRO was not detected in MTZS (n=557) isolates, but was found in all MTZR (MIC ≥ 4 as determined by agar dilution) strains including those analyzed by WGS. pCD-METRO was found in various RT010 isolates from the reference laboratory (Netherlands), RT010s from Spain, Czech Republic and Germany, and in a a Polish RT027. These results indicate that pCD-METRO can be detected in toxigenic and non-toxigenic isolates from human- and animal origin, including epidemic types.

A ~25 fold increase in MIC value was attributed to the presence of pCD-METRO in 630Δ*erm*.
We identified a 7 kb unique plasmid capable of conferring metronidazole resistance in *C. difficile*, originating from an bacterial unknown donor. The plasmid is globally disseminated and occurs in both non-toxigenic- and toxigenic strains, including epidemic types. This is the first report of a clinically relevant phenotype associated with plasmid carriage in *C. difficile*. Prospective screening of clinical samples for pCD-METRO in the national reference laboratory could be implemented.

1. Ooijevaar, R.E., et al., Update of treatment algorithms for *Clostridium difficile* infection. Clin Microbiol Infect, 2018. 24(5): p. 452-462.

Conflict of interest: No

O 12

Transcriptomic, proteomic and functional analysis of the plasmid pCS1-1 during spore formation in *Clostridium sordellii*

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Clostridial plasmids have been shown to carry antibiotic resistance determinants and virulence factors, resulting in the ability for these mobile genetic elements to contribute to pathogenesis. While many clostridial strains carry plasmids, the role of plasmids in spore formation has not been studied. Using *Clostridium sordellii* as our model spore-forming organism, we have focussed on the 103kb toxin-encoding conjugative plasmid, pCS1-1, to investigate if plasmids contribute to, or are influenced by, spore formation.

We performed an RNA-Seq time course analysis on *C. sordellii* strain ATCC9714 during sporulation to identify the transcriptional changes to plasmid genes during early and late spore formation. Of the 89 predicted genes present on pCS1-1, we have identified three genes (*gll*, *hyp13* and *asrA*) that are upregulated during spore formation, compared to vegetative cell growth. One of these genes, *asrA*, was insertionally inactivated using the TargeTron mutagenesis system and the resultant mutant characterised. The plasmid-encoded *asrA* gene was found to influence the temporal progression of sporulation, with the mutant *asrATT* displaying a delayed sporulation phenotype.

A proteomic analysis of mature *C. sordellii* spores was also performed. Purified spores were cryomilled to disrupt their integrity, and mass spectrometry was used to identify the plasmid-encoded proteins that contribute to the mature spore proteome. Five of the potential 89 proteins predicted to be encoded by pCS1-1 were also found to be present in the mature spore proteome. The function of these plasmid-encoded proteins identified in our proteomics study is currently under investigation.

It has previously been assumed that while plasmids are important during vegetative growth, they are not active or relevant during the process of spore formation. These results have shown, through a combination of transcriptomic and proteomic analyses, that plasmid-encoded elements are active during sporulation. In addition to this, we have demonstrated genetically that *asrA* on pCS1-1 contributes to the progression of sporulation. This is the first time that a plasmid-encoded gene has been shown to modulate the sporulation process in any spore-forming bacterium. This work supports the need for further research into the role of plasmids throughout all life stages of the clostridia.

Conflict of interest: No

O 13

The serine proteases CspA and CspC are essential for *Clostridium perfringens* SM101 spore germination through activating SleC and cortex hydrolysis

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Clostridium perfringens SM101 genome encode three serine proteases (CspA, CspB, and CspC), and CspB converts the inactive pro-SleC into active SleC, an enzyme essential for degradation of the peptidoglycan cortex during spore germination. In this study, the expression of *cspA* and *cspC*, as well as the germination and colony formation by spores of *cspAC* and cspC mutants of strain SM101, were assessed. We demonstrated that, 1) the cspA and cspC genes were expressed as a bi-cistronic operon only during sporulation in the mother cell compartment of SM101; 2) both *cspAC* and *cspC* spores were unable to germinate significantly with either KCl, L-glutamine, brain heart infusion broth, or a 1:1 chelate of Ca^{2+} and dipicolinic acid (DPA); 3) in consistence with germination results, both *cspAC* and *cspC* spores were defective in normal DPA release; 4) the colony formation by *cspAC* and *cspC* spores was $10\Lambda^{6}$-fold lower than that of wild-type spores, although these spores yielded wild-type level colony formation on plates containing lysozyme; 5) no processing of inactive pro-SleC into active SleC was observed in *cspAC* and *cspC* spores during germination; and Finally, 6) the defects in germination, DPA release, colony formation and SleC processing in *cspAC* and *cspC* spores were complemented by wild-type *cspAC* operon. Collectively, these results indicate that both CspA and CspC are essential for *C. perfringens* spore germination through activating SleC and cortex hydrolysis.

Conflict of interest: No

O 14

Structure and assembly of S-layer in *C. difficile*

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Many bacteria are coated with a proteinaceous surface layer - S-layer - which is formed by the self-assembly of monomeric proteins into a regularly spaced, two-dimensional array. S-layers are implicated in growth and survival, and their many functions include the maintenance of cell integrity, enzyme display and, in pathogens and commensals, interaction with the host and its immune system. Despite its key role in many bacteria, structural and functional details of complete S-layer proteins are still lacking.

In *C. difficile*, the S-layer has been linked to several key pathogenicity traits including colonisation and activation of inflammatory, immune responses. The principal S-layer protein, SlpA, displays sequence diversity between strains, with 13 antigenically distinct types identified and it was recently shown that lack of SlpA results in strains unable to cause disease in hamsters.

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). The SLPs form a high-affinity heterodimer that self-assembles to produce the S-layer, with 3 cell wall binding (CWB2) motifs in the HMW providing the non-covalent anchor to the cell wall, and the LMW presented to the external environment.

Here, we report the complete structure of SlpA and a proposed model for S-layer assembly. Using a combination of X-ray crystallography and 2D electron diffraction, we determined the first complete crystal structure of an S-layer protein in a pathogenic bacterium and the overall assembly of the S-layer. This informed our work to investigate the role of certain domains of SlpA in *C. difficile* pathogenicity.

We obtained diffracting crystals of the whole LMW/HMW complex from different *C. difficile* strains and crystals of different subdomains, including the two interacting domains (LID/HID). Combining S-SAD phases (I23, Diamond Light Source), determined structures of the subdomains and data from different SlpA

proteins allowed us to solve the full structure of different SlpA cassette types. We also determined the structure of the native *C. difficile* S-layer from different strains to 20 Å using electron crystallography. Fitting the crystal and EM models allowed us to develop the first model for the assembly and structure of the mature S-layer in *C. difficile*. This model was then tested by deleting key domains and investigating their role in structure, assembly and, importantly, in pathogenicity. Surprisingly, deleting exposed domains of the LMW protein resulted in a strain that has attenuated virulence in the mouse model, despite similar colonisation, sporulation and toxin levels, providing the first direct evidence that SlpA plays a role in disease severity.
This work presents the first structural and functional model of a complete S-layer and paves the way for exploring these structures as potential therapeutic targets.

Conflict of interest: No

O 15

Colitis-induced Th17 cells increase the risk for severe subsequent *Clostridium difficile* infection

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Clostridium difficile infection (CDI) is the number one hospital-acquired infection in the United States. CDI is more common and severe in inflammatory bowel disease patients. Here, we studied the mechanism by which prior colitis exacerbates CDI. Mice were given dextran sulfate sodium (DSS) colitis, recovered for 2 weeks, and then were infected with *C. difficile*. Mortality and CDI severity were increased in DSS-treated mice compared to controls. Severe CDI is dependent on CD4+ T cells, which persist after colitis-associated inflammation subsides. Adoptive transfer of Th17 cells to naive mice is sufficient to increase CDI-associated mortality through elevated IL-17 production. Finally, in humans, the Th17 cytokines IL-6 and IL-23 associate with severe CDI, and patients with high serum IL-6 are 7.6 times more likely to die post infection. These findings establish a central role for Th17 cells in CDI pathogenesis following colitis and identify them as a potential target for preventing severe disease

Conflict of interest: No

O 16

The role of dendritic cells in *Clostridium difficile* infection

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C. difficile colonization and toxin production can lead to a wide range of symptoms from diarrhea to pseudomembranous colitis, systemic disease and death. The host immune status plays an important role in protection against *C. difficile* infection (CDI), especially the dendritic cells (DCs) as a bridge to connect innate and adaptive immunity. However, their role during CDI has not been characterized. In this study, we adopted a murine model of transgenic mice Tg-CD11c-DTR/EGFP (Tg), whose DCs can be conditionally depleted by injection of diphtheria toxin (DT), to investigate the role of DCs in CDI. We found that DT-injected Tg mice were more vulnerable to the CDI, compared with the PBS-injected Tg mice. DT-injection significantly depleted DCs, impaired the host capability to clear off *C. difficile* in the intestine, and increased the infiltration of other bacteria into organs. DC deletion did not seem to affect intestinal microbiota, nor neutrophil infiltration into lumen, but reduced macrophage infiltration into intestinal lumen. Interestingly, DC depletion impaired the intestinal barrier integrity, which could partially contribute to the increased infiltration of intestinal bacteria to organs, and severity of CDI. Transferring DCs to wild-type mice conferred host resistance to CDI. These data clearly demonstrate a protective role of DCs against CDI.

Conflict of interest: No

O 17

Host immunity modulates efficacy of fecal microbiota transplantation treatment of *Clostridium difficile* infection

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Clostridium difficile is an opportunistic pathogen that infects the large intestine following perturbation of the intestinal microbiota causing epithelial damage and debilitating, potentially fatal, colitis. Current antibiotic treatment options result in high recurrence rates, highlighting the need to identify alternative approaches to control this disease. Fecal microbiota transplantation (FMT) is clinically proven to efficiently clear recurrent *C. difficile* infection. Despite remarkable efficacy, implementation of FMT therapy is limited due to inadequate understanding of FMT mechanism of action. We use a murine model of persistent *C. difficile* infection to demonstrate a critical role for the host's immune system in supporting efficacy of FMT. Immunodeficient Rag1-/- and littermate Rag1HET mice infected with *C. difficile* exhibit comparable establishment of persistent infection with no difference in bacterial burden or toxin levels in the cecum. Following FMT, Rag1HET mice resolve *C. difficile* infection while cohoused Rag1-/- mice fail to clear the infection. Analysis of the intestinal microbial communities following FMT demonstrated that the microbiota of Rag1HET mice assimilate toward the composition of the FMT donor, while Rag1-/- mice exhibit impaired engraftment of bacterial species associated with 2° bile acid biosynthesis. Conversion of 1° bile acids to 2° bile acids in the cecum drives resolution of *C. difficile*. Metabolomic analysis of cecal content from Rag1HET mice following FMT revealed restoration of 2° bile acids while the cecal content from FMT-treated Rag1-/- mice remain comprised of 1° bile acids. These data support a role for host immunity in determining efficacy of microbiota-based therapeutics.

Conflict of interest: No

O 18

Evaluating whether claudin-1 affects the action of C. *perfringens enterotoxin* on Caco-2 cells

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Clostridium perfringens can produce numerous toxins and its broad-ranging pathogenesis is associated with the many toxins that it synthesizes. One of the toxins produced is the enterotoxin (CPE), which is important in medicine, veterinary sciences and for translational applications. For example, CPE production is required when type F strains cause human food poisoning. In addition, CPE is necessary for type F strains to cause non-food borne gastrointestinal diseases of humans, such as antibiotic-associated diarrhea. CPE is a pore-forming toxin that belongs to the aerolysin toxin family. CPE has a complicated mode of action that starts with its binding to claudin receptors, such as claudin-3 and -4 (tight junction proteins), forming a small complex (90kDa). This small complex then oligomerizes into a ~450kDa complex to create a prepore, followed by insertion of b-hairpins from CPE to form a b-barrel pore named CH-1 (CPE hexamer-1).
There are ~27 claudin proteins but CPE binds to different claudins with very variable affinity. Previous studies using co-immunopercipitation and electroelution techniques showed that the small and CH-1 complexes contain, at minimum, CPE and both receptor and non-receptor claudins (1). One non-receptor claudin is claudin-1, which binds CPE poorly or not at all. However, claudin-1 is present in both complexes. The main objective of this study was to evaluate if claudin-1 affects the action of CPE on Caco-2 cells. For this purpose, claudin-1 null mutant and complementing Caco-2 cell lines were generated using CRISPR-cas9 and stable transfection approaches, respectively. The claudin-1 null mutant and complemented cells were validated by PCR and western blot. Subsequently, integrity and permeability of these cells as well as their sensitivity to CPE toxin and the stabilty of CH-1 complex were evaluated. Results indicated that, in the claudin-1 null mutant, paracellular permibility was highly enhanced. When cytotoxicity was measured using an LDH-release assay, the claudin-1 null mutant was markedly more sensitive to CPE compared to wild-type Caco-2 cells. In addition, we found that CPE forms more small complex in claudin-1 null mutant than wild type cells. This finding suggests that more CPE binds to cell surface receptors of claudin-1 mutant cells than to wild-type Caco-2 cells. In addition, the CH-1 complex formed by CPE on claudin-1 null mutant cell lines was less stable

than the CH-1 complex of wild type cells, suggesting claudin-1 is important for stability of CH-1 complex. These results indicate that even nonreceptor claudins can influence CPE action.

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Conflict of interest: No

O 19

The role of siderophores in iron acquisition during *Clostridioides difficile* pathogenesis

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Clostridioides difficile (Cd) is the leading cause of antibiotic associated diarrhea. Disruption of the normal microbiota by antibiotics renders the gut susceptible to Cd infection. During colonization Cd must obtain essential nutrients for growth, including iron, which is used both by host cells and bacteria for many cell processes. Very little free iron is available in a mammalian host due to many iron storage mechanisms. Bacterial pathogens have evolved numerous mechanisms for acquiring iron, including small high affinity molecules called siderophores. We have shown in iron depleted media (IDM), which restricts growth, that siderophores (ferrichrome, enterobactin, salmochelin and yersiniabactin) restore growth to the level of 2 μM iron supplementation. Considering that many commensal bacteria likely produce siderophores, we developed an assay to determine if the siderophore distribution changes upon antibiotic treatment. Intestinal contents from mice before and after treatment with cefoperazone were assessed by mass spectrometry to determine which siderophores are present. Pathogenic bacteria can gain a competitive advantage if they produce their own local pool of siderophore. However, the genomes of most Cd isolates encode genes for putative siderophore transporters, but lack known siderophore biosynthetic genes. Interestingly, analysis of sequenced Cd isolates revealed genes for biosynthesis of the siderophore yersiniabactin in a subset of isolates. We performed RNA-seq on one yersiniabactin positive strain (VPI10463) grown in rich media (BHIS) or iron depleted media (BHIS + deferoxamine). The genes for yersiniabactin biosynthesis and the predicted transporter YbtP/Q were highly induced. We hypothesize that yersiniabactin plays a role in Cd pathogenesis and may contribute to the increased disease severity observed for some of these isolates. To test this hypothesis, we are examining the role of siderophore acquired iron from Cd produced yersiniabactin vs xenosiderophores. To determine if Cd produces yersiniabactin in vivo, germ-free mice were infected with Cd strain VPI10463 and intestinal contents were

evaluated for yersiniabactin. In addition, we are working to make isogenic mutants lacking the yersiniabactin biosynthesis pathway and the putative siderophore transporters. Current methods allow for genetic manipulation of one or two strains, but have not worked in strains harboring the genes for yersiniabactin biosynthesis. Therefore, CRISPRi plasmids were constructed to knockdown expression of these genes and examine their role in pathogenesis. We are also interested in how *C. difficile* uses the three predicted ABC transporters to import siderophores. While only a subset of strains have genes to produce yersiniabactin, strains that do not encode for yersiniabactin are able to utilize it as an iron source. To determine if these transporters can import only a single siderophore, or multiple siderophores, we purified the extracellular domain of the siderophore binding proteins and siderophore binding will be determined by isothermal titration colometry. Greater knowledge about the role of siderophores in Cd iron acquisition and differences in the pathogenesis of different Cd strains will lead to improved treatment options.

Conflict of interest: No

O 20

The detection and isolation of ε-toxin producing *Clostridium perfringens* from Multiple Sclerosis patients

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The spore-forming anaerobe *Clostridium perfringens* is a multi-toxin producer, ubiquitously found in nature, including the GI tracts of humans and animals. Strains produce a variety of toxins for which they are categorized into 7 toxinotypes, where types B and D produce and secrete ε-toxin (Etx), the most potent of all ~20 toxins present in the pan-genome. Etx-producing strains cause neurological disorders and death in ruminants and other animals, but are rarely associated with humans. However, experimental analysis shows that Etx rapidly disrupts the blood brain barrier and targets oligodendrocytes leading to demyelination, two hallmarks of newly formed Multiple Sclerosis (MS) lesions, an inflammatory demyelinating disease of the central nervous system in humans. Therefore, we hypothesize that in genetically susceptible individuals, Etx produced by *C. perfringens* types B and D is a possible environmental trigger of MS. Fecal samples collected from 24 healthy control and 24 MS subjects were used to test this and were interrogated for the presence of Etx producing *C. perfringens*. This was achieved by culturing the fecal microbiota in rapid perfringens media, where total DNA was extracted and subsequently toxinotyped via PCR. Culture supernatant was screened for the presence of Etx via a highly sensitive sandwich ELISA specific for Etx. We determined that while no Etx producing *C. perfringens* was detected in healthy controls, 21% of the MS patients contained Etx producing *C. perfringens* in their feces (4 type D and 1 type B). Further, we were able to isolate the first pure type D strain from a patient suffering from MS. Through pulse field gel electrophoresis, we determined that the isolate carries one plasmid ~65 kb in length. We then generated a high quality draft genome via a hybrid assembly approach leveraging long, single-molecule nanopore reads and short, high-quality Illumina reads, which phased and annotated the etx gene providing insight into the isolate's physiology and biology, and the genomic context of the etx gene. In support of our hypothesis, the type D isolate culture supernatant induced demyelination of cerebellar organotypic slice cultures in an Etx-dependent manner. Our findings suggest there is a link between Etx producing *C. perfringens* and MS, and establishes the basis

for further rigorous investigations into the nature of this relationship, and more sensitive methods for detecting *C. perfringens* in MS patients.

Conflict of interest: No

O 21

Differential involvement of S100B/RAGE signaling in *Clostridioides difficile* toxin-induced inflammation and apoptosis in enteric glial cells

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Clostridioides difficile infection (CDI) continues to be the most common cause of antibiotic-associated diarrhea. The enteric glial cells (EGC) play a pivotal homeostatic role in enteric nervous system-mediated intestinal motility, inflammation and barrier function. We investigated EGC-derived S100B activity in CDI and the involvement of S100B/RAGE signaling in TcdA- and TcdB-induced inflammation and apoptosis in EGCs.
Expression of S100B was examined by immunohistochemistry in colonic biopsies from patients with active CDI, cecal tissues of VPI10463-infected mice and mouse ileal loop treated with TcdA. Rat EGC line CRL2690 (ATCC) was incubated with TcdA and TcdB to evaluate cell viability through MTT assay, cell morphology by light microscopy and apoptosis by a live-cell real-time assay. Gene expression of S100B, RAGE and IL-6 were performed by qPCR. Extracellular S100B was measured by ELISA. NFκB and STAT3 activation were evaluated by immunofluorescence. To investigate the role of S100B/RAGE signaling pathways, we used S100B inhibitor pentamidine, RAGE antagonist FPSZM1, PI3K inhibitor LY294002 and STAT 3 inhibitor galiellalactone.
S100B expression was significantly increased in mouse ileal loop tissue treated with TcdA, in cecal tissues from *C. difficile*-infected mice, as well as, in colonic biopsies from patients with CDI compared to controls. TcdA and TcdB significantly decreased EGC viability, induced cell rounding and apoptosis, induced NFκB and STAT-3 activation, upregulated S100B and IL-6 gene expression, and increased S100B release. Pentamidine suppressed TcdA-, but not TcdB-induced S100B and IL-6 expression. Blockade of S100B receptor RAGE significantly decreased TcdB and TcdA-induced IL-6 and S100B gene expression in EGCs, as well as, TcdA-induced apoptosis. Blockade of NFκB by PI3K inhibition, decreased IL-6 expression, but not S100B, induced by both toxins. Inhibition of STAT-3,

significantly reduced TcdB and TcdA-induced S100B gene expression, as well as its release, and apoptosis on EGCs.
Our findings suggest that S100B/RAGE/PI3K/NFkB signaling is involved in TcdA and TcdB-induced IL-6. In addition, S100B/RAGE/STAT-3 pathway is involved in TcdB-induced S100B and IL-6 expression and TcdA-induced S100B expression and apoptosis. However, apoptosis induced by TcdB appears to be mediated by S100B/STAT-3 in a RAGE-independent manner.

Conflict of interest: No

O 22

Anti-sporulation strategies targeting *Clostridioides difficile* and other spore-forming bacterial pathogens

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Spore-forming bacteria include the devastating human and animal pathogen *Clostridioides difficile,* the food spoilage pathogen *Bacillus cereus* and the agent for bioterrorism *Bacillus anthracis*. Spores are the infectious particles of these pathogens and their resistant and unique structure makes them difficult to eradicate. Their persistence properties enable the spread of disease, resulting in fatalities and economic devastation in environments such as health care settings, the food industry and public spaces in the case of weaponised anthrax. Despite this major burden, there are no strategies to prevent spore production.
C. difficile is of considerable medical interest due to the disease burden and global challenge of managing the consequences of infection. Spores are a crucial mediator of *C. difficile* disease initiation, dissemination and re-infection and are highly resistant to current therapeutics which do not target sporulation.
Here we report the novel finding that a group of compounds (DL01, DL02 and DL03) can directly impact *C. difficile* spore production by inactivating spore-specific proteins. Through TEM imaging we observed that DL compounds block the early stages of sporulation. We also found that this sporulation inhibition phenotype is generic using the spore-forming pathogens *Paeniclostridium sordellii*, *Bacillus subtilis* and *B. cereus*, a close relative of *B. anthracis*, confirming that that DL compounds have broad applicability as an anti-sporulation strategy.
The major anti-sporulation molecular target of the DL compounds was identified as a spore-specific protein, YS01. Sporulation assays using a YS01 mutant confirmed that this protein has an essential role in sporulation. YS01 is also found in other spore-forming bacteria, including *B. anthracis* and *B. cereus*. Through our binding assays we show that the DL compounds target YS01 in all of these pathogens.
Of clinical relevance, we also found that co-treatment of mice with the DL compound, DL02, and the current primary *C. difficile* treatment vancomycin prevented disease re-infection. This is a highly significant result as up to 35% of patients experience *C. difficile* re-infection after treatment ceases. Our findings could therefore directly and immediately impact *C. difficile* infection treatment.

Importantly, our results also provide new insights into disease prevention through targeting of the sporulation process. They also provide evidence of potential new anti-sporulation targets that could significantly advance drug development for other important spore-forming pathogens that are problematic in the food industry (*B. cereus*) and as bioterrorism agents (*B. anthracis*).

Conflict of interest: No

O 23

Strain Colonization of a defined bacterial consortium and eestoration of the microbiota and metabolite pool of healthy volunteers

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Alterations of the human gut microbiota are associated with infections by opportunistic pathogens like *Clostridioides difficile* (*C. difficile*). Reduction in *C. difficile* infection recurrence rate has been shown with fecal microbiota transplantation (FMT); however, FMT carries inherent risks and variability. Vedanta Biosciences is developing VE303, a live biotherapeutic product (LBP) for the prevention of recurrent *C. difficile* infection (rCDI). VE303 is a rationally-defined bacterial consortium that consists of 8 purified, clonal strains belonging to *Clostridium* clusters IV, XIVa, and XVII. The strains were isolated from the fecal matter of healthy volunteers, banked as pure cultures, and manufactured under cGMP conditions. Safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of VE303 were assessed in healthy volunteers (N=33). Increasing doses of VE303 were administered with or without a 5-day course of oral vancomycin. Fecal samples were collected longitudinally up to 6 months and metagenomic sequencing was performed on the Illumina platform to quantify the colonization of the VE303 strains over time (Pharmacokinetics, PK) and their impact on the recovery of the resident gut microbiota and the levels of short chain fatty acids (SCFA) and bile acids (BA) (Pharmacodynamics, PD) following vancomycin exposure. A scalable bioinformatic method that utilizes unique marker sequences for each VE303 strain was developed to precisely define PK by differentiating the members of the LBP from resident strains. VE303 administration post-vancomycin was safe and well tolerated across all dose levels. Dosing conditions were identified where the VE303 strains rapidly increased in abundance and were detected at least 6 months after administration of the consortium. VE303 also led to an enhancement in the gut microbiota recovery post-vancomycin, demonstrated by an accelerated increase in *Clostridium* and Bacteroidetes species and reduced Proteobacteria. VE303 strain colonization was also associated with expedited recovery of SCFAs and secondary BAs measured in the stool post-vancomycin

administration. Collectively, these data indicate for the first time that an LBP based on a rationally-defined bacterial consortium is safe, can durably colonize the gut, and is capable of promoting intestinal homeostasis of human recipients.

Conflict of interest: Yes
Disclosures: JMN, RM, JH, BK, DB, and BR are employees of Vedanta Biosciences.

O 24

Fidaxomicin association with the exosporium of *Clostridium difficile* spores

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Fidaxomicin has demonstrated novel pharmacologic effects on *C. 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 ≈10^7CFU/mL. One hundred µl of spores and 100µg/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. Exosporium removal was done by treating the samples with either 1% sarkosyl-proteinase K or trypsine solutions at 300rpm for two hours followed by five washes in PBS. The samples were stained with FM4-64 (excitation/emission ~515/640) to visualize the surface layer 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 of *C. difficile*: 027; 012 and 078 because of the variations observed in their exosporium thickness, as well as *B. subtilis* that doesn’t display an exosporium. Bodipy-fidaxomicin surrounding the *C. difficile* spores was observed for all *C. difficile* ribotypes but not for *B. subtilis*. Also when the *C. difficile* spores were treated for exosporium removal after incubation with fidaxomicin, the association was lost. If the exosporium was removed before the co-incubation spores-fidaxomcin, the association was weaker. In conclusion, this study showed a direct and strong association between fidaxomicin and *C. difficile* spores’ exosporium may be due to compound specificity rather than hydrophobicity according to the lack of interaction with *B. subtilis* spores that are

highly hydrophobic. All in all, these data provide advanced insight regarding the anti-spore properties of this compound.

Babakhani et al. 2012; Chilton et al. 2016; Paredes-Sabja et al. 2014; Wiencek et al. 1990
Funding: Merck & Co., Inc.

Conflict of interest: No

O 25

Divergent host and metabolic responses to fecal microbiota transplantation from different hosts in a mouse model of *Clostridium difficile* infection

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Microbial therapeutics have great promise in treatment of various infectious diseases and other gastrointestinal conditions. Despite the success of one such treatment method, fecal microbiota transplantation (FMT), in treating recurrent *Clostridium difficile* infection (CDI), it is still unclear how a certain environment influences the ability of a microbial community to produce metabolites hypothesized to combat *C. difficile*. In a mouse model of recurrent CDI, we observed that fecal material from healthy human donors (hFMT) that had previously been used to successfully treat patients with recurrent CDI was not sufficient to clear *C. difficile* in mice. This was in contrast to fecal material from healthy mice (mFMT), which induced *C. difficile* clearance in our model. Initial 16S rRNA gene-based sequencing revealed that the fecal microbiota community in mice treated with both hFMT and mFMT contained diverse members of Clostridiales and Bacteroidales, microbes commonly associated with recovery of the microbiota following FMT. Similar microbes from hFMT were also capable of colonizing germ-free mice, suggesting that the inability of hFMT to clear *C. difficile* was not due to the inability of hFMT microbes to colonize conventional, antibiotic-treated mice with CDI. To compare metabolic capabilities of mFMT- or hFMT-treated mice, we measured bile acids and short chain fatty acids (SCFAs) following treatment. We observed that each treatment induced disparate metabolic environments; while hFMT-treated mice were capable of producing some secondary bile acids and high levels of the SCFAs acetate and propionate, hFMT-treated mice produced low levels of butyrate. Host transcriptome analysis revealed major differences in response to hFMT and mFMT suggesting that hFMT in mice may disrupt host-microbiota homeostasis. We observed decreased expression of genes involved in the production or regulation of defensins, interferons, and other innate immune pathways in response to mFMT during clearance of *C. difficile* compared to treatment with hFMT or no treatment. Several metabolic and signaling pathways were also differentially expressed in response to mFMT or hFMT, such as signaling by G-protein-coupled receptors and expression of enzymes involved in fatty acid

cleavage. Together, these data suggest disparate metabolic and host-modifying properties of microbial communities adapted to different hosts, even if these communities hypothetically have the ability to produce similar functions. In addition to identifying metabolites critical to clearance of *C. difficile*, future aims include identifying what metabolic or host factors stimulate a microbial input to produce distinct metabolites.

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Conflict of interest: No

O 26

Microbiota-regulated IL-33 restricts the severity of *Clostridium difficile* infection via Type 2 Innate Lymphoid Cell

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Emerging evidence indicates gut immunity to *C. difficile* is a delicate balance between protection and pathology. To identify potential therapeutic host immune targets, we conducted a transcriptome analysis of host genes altered by hypervirulent infection and identified interleukin-33 (IL-33) as a gene upregulated in response to infection. Using a murine model, we show that both endogenous IL-33 and exogenous IL-33 treatment prevent mortality, weight-loss and epithelial disruption during hypervirulent *C. difficile* infection and protection was broad against both epidemic and non-epidemic *C. difficile* infection. IL-33 mediated protection required type-2 innate lymphoid cells (ILC2s) and adoptive transfer of purified ILC2s was sufficient to mitigate CDI- associated mortality and weight-loss. Furthermore, dysregulated IL-33 signaling via the soluble IL-33 decoy receptor (sST2) predicted disease severity and mortality in human patients. Lastly, colonic IL-33 expression appears to be regulated by the microbiota as antibiotic- depletion of IL-33 was rescued with mouse fecal microbiota transplant (FMT) and a human fecal spore preparation (HSP). Thus, IL-33 signaling is a novel therapeutic pathway for during *C. difficile* infection which can potentially be targeted with rationally designed microbial therapies.

Conflict of interest: Yes

Disclosures: WP is a consultant for TechLab, Inc.

P 1

Novel Chimeric Protein Vaccines against *Clostridium difficile* Infection

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Clostridium difficile infection (CDI) is the leading cause of world-wide nosocomial acquired diarrhea in adults. Active vaccination is generally accepted as a logical and cost-effective approach to prevent CDI. In this paper, we have generated two novel chimeric proteins; one designated Tcd169, comprised of the glucosyltransferase domain (GT), the cysteine proteinase domain (CPD) and receptor binding domain (RBD) of TcdB, and the receptor binding domain of TcdA; the other one designated Tcd169Fl, which contains Samlnonella typhimurium flagellin (sFliC) and Tcd169. Both proteins were expressed in and purified from Bacillus megaterium. Point mutations were made in the GT (W102A, D288N) and CPD (C698) of TcdB to ensure that Tcd169 and Tcd169Fl were atoxic. Immunization with Tcd169 or Tcd169Fl induced protective immunity against TcdA/TcdB challenge through intraperitoneal injection, also provided mice full protection against infection with a hyper-virulent *C. difficile* strain (BI/NAP1/027). In addition, inclusion of sFlic in the fusion protein (Tcd169Fl) enhanced its protective immunity against toxin challenge, reduced *C. difficile* numbers in feces from Tcd169Fl-immunized mice infected *C. difficile*. Our data show that Tcd169 and Tcd169Fl fusion proteins may represent alternative vaccine candidates against CDI.

Conflict of interest: No

P 2

A Novel Bacteriophage Lysin-human Defensin Fusion Protein is Effective in Treatment of *Clostridioides difficile* Infection in Mice

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Clostridioides difficile is the leading cause of worldwide antibiotics-associated diarrhea. In this study, we report the construction and evaluation of a novel bacteriophage lysin-human defensin fusion protein targeting *C. difficile*. The fusion protein, designated LHD, is composed of two parts connected by a 3-repeating unit linker "(GGGGS)3": the catalytic domain of a lysin protein from a *C. difficile* bacteriophage phiC2 (LCD) and the functional domain of a human defensin protein HD5. Lytic assays showed that LHD protein had a potent lytic activity against different types of clinical *C. difficile* strains, including the epidemic 027, 078, 012, and 087 strains. The minimum inhibitory concentration (MIC) of LHD was 0.78 µg/ml, which was lower than the MIC of the protein LCD (1.56 µg/ml), and the MICs of metronidazole (4 µg/ml) and vancomycin (4 µg/ml). In addition, the LHD protein could lyse C. different strains in different pHs (6.0, 7.0, and 8.0). Evaluation of LHD potency in vivo using mouse model of *C. difficile* infection (CDI) showed that administration of the LHD protein (twice daily for seven days) was effective in mitigating the symptoms and reducing the death from CDI. Treatment with LHD also significantly decreased the number of *C. difficile* spores and the toxin level in feces from the infected mice. Our data suggest that this novel lysin-human defensin fusion protein has a potential on CDI control.

Conflict of interest: No

P 4

Phylogenomic analysis of gastroenteritis-associated *Clostridium perfringens* identifies isogenic strains in multiple outbreaks and novel virulence-related features

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Clostridium perfringens is a major enteric pathogen known to cause gastroenteritis in humans. Although major outbreak cases are frequently reported, to date no Whole Genome Sequencing (WGS) based studies have been performed to understand the genomic epidemiology and virulence gene content of *C. perfringens*-associated outbreak strains.
Methods: We have applied both genomic and phylogenetic analyses on a sub-set of 109 newly-sequenced *C. perfringens* strains isolated from gastroenteritis-associated disease cases (including food-poisoning and care-home diarrhoea) from England and Wales between 2011-2017 to probe virulence profiles including toxin and antimicrobial resistance (AMR) genes, plasmid features and genomic epidemiology of these case isolates.
Major findings: Our data identified that highly-similar *C. perfringens* strains were associated with 9 care home-associated individual outbreaks over a 5-year interval, indicating potential common sources linked to these distinct outbreaks. Enterotoxin gene *cpe* was encoded in all but 4 isolates (96.4% type-F strains), and it was further determined that virulence plasmids encoding cpe were extensively distributed in the isolates (97% care-home isolates carry pCPF5603 plasmid; 60% food-poisoning isolates carry pCPF4969 plasmid). Further virulence factors, such as β2-toxin, were enriched in these isolates (46.7%). Phage proteins were also commonly identified, with additional analysis indicating phages may contribute to spread of virulence determinants.
This study of gastroenteritis-associated *C. perfringens* highlights the genotypic and epidemiological relatedness of a large collection of *C. perfringens* strains isolated from gastroenteritis-associated cases from across the United Kingdom and Wales. Key points revealed include the potential circulation of disease-associated strains, and impact of cpe-encoding-plasmid disseminations, linked to outbreak cases.

Conflict of interest: No

P 5

Mutational analysis of a multidrug efflux transporter in *Clostridium difficile*

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Antibiotic resistance in *Clostridium difficile* is one of the most important factors to promote the infection, level of severity and incidence of recurrence. One of the mechanisms that has been linked with antibiotic resistance in this pathogen is efflux pump through the ATP-binding-cassette (ABC) transporter. An ABC transporter possesses transmembrane domains that function in substrate translocation, and nucleotide binding domains, which supply the energy through ATP-hydrolysis. Our previous finding revealed the association of CD2068 with multidrug resistance in *C. difficile*. Reduction in antibiotic susceptibility was also observed when the recombinant CD2068 was heterologously expressed in *Escherichia coli*, suggesting the role for this protein in the resistance mechanism against multiple drugs. To further understand the molecular mechanism and function of CD2068, mutational analyses were performed. The results revealed that Lys40, Gly159 and Gly442 but not Thr358 in CD2068 were critical for the function as mutant strains exhibited higher sensitivity to the tested antibiotics. Our finding indicated that the first Walker A motif and two ABC signature motifs, but not the second Walker A play an important role in antibiotic resistance. Extensive mutational analysis was also performed by random mutagenesis using error-prone polymerase chain reaction to investigate other essential amino acid residues for CD2068 function.

Conflict of interest: No

P 6/FL 8

A restriction-modification system associated with phosphorothioation of DNA in *Clostridium perfringens*

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Major advances in *Clostridium perfringens* genetics have followed the development of electroporation-induced transformation. However, some strains are still refractory to transformation, presumably owing to their restriction-modification (R-M) systems. Herein, we focused on phosphorothioation (PT) of DNA, in which sulphur replaces a nonbridging phosphate oxygen, as occurs in one of the R-M systems in Gram-negative bacteria. Among *C. perfringens* strains causing foodborne illness, NCTC8239, W5837, and W09-505 remain unamenable to genetic manipulation. We found that the genome of NCTC8239 includes the gene cassette dndABCDE and *dptFGH* that is predicted to function for PT modification and restriction. Oxidant-dependent degradation of genomic DNA suggested that the genome of NCTC8239 is PT-modified. When foreign DNA was PT-modified using a plasmid expressing PT modification enzymes (DptBCDE) from *Salmonella enterica*, the efficiency of transformation into NCTC8239 was significantly higher than when using an unmodified plasmid. A dptG-null mutant exhibited significantly higher transformation efficiency with unmodified foreign DNA than the wild-type strain. Furthermore, the unmodified plasmid was transformed into the mutant as efficiently as PT-modified plasmid. Collectively, these results suggested that PT participates in an R-M system in *C. perfringens*. To our knowledge, this is the first study suggesting PT-dependent R-M system capable of protection against the invasion of unmodified foreign DNA in low G-C Gram-positive bacteria.

Conflict of interest: No

P 7

Commercial vaccines containing epsilon and botulinum toxoids do not induce one-year lasting humoral response in cattle

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Among clostridial diseases, enterotoxaemia, caused by *Clostridium perfringens* type D, and botulism, caused by *Clostridium botulinum* types C and D, represent serious risks for cattle. Eradication of clostridial diseases is impractical and the treatment of affected animals is commonly not feasible, so the prevention and control of these diseases is based on the systematic vaccination of herds. The scientific literature and most vaccine manufacturers recommend vaccination of calves around 4 months of age, followed by a booster after 4 to 6 weeks and annual revaccination of adults. Anyway, it is still unknown if these commercial vaccines would induce a humoral response in cattle that lasts until the next vaccination, after around one year. Thus, the aim of this study was to evaluate the titers of neutralizing antibodies in cattle inoculated with multivalent commercial clostridial vaccines containing *C. botulinum* type C (BoNTC), *C. botulinum* type D (BoNTD), and *C. perfringens* epsilon (ETX) toxoids for a period of one year. Cattle (*Bos taurus*), aged 4 to 6 months and not previously immunized, were vaccinated under four different protocols at days 0 and 30 and followed over one year. Individual serum titration was performed by a serum neutralization test in mice or in MDCK cells. A significant reduction in the titers was observed 60 days after the booster vaccination in all groups and for all antigens tested. These reduction of the mean titers against BoNTC was slightly slower than those induced by ETX. BoNTD prompted the highest humoral response at peak, and consequently, the longest lasting titers. Still, antibodies against BoNTD were last detected at 180 days, while antibodies against BoNTC and ETX were last detected at 120 and 60 days, respectively. According to the manufacturers´ instructions of the tested vaccines and also the current literature, the revaccination for clostridial diseases in cattle would be indicated one year after the booster dose. According to the results of this study, these animals would be unprotected for several months until revaccination. The results shown in this present work encourage discussion on the actual protocols recommended for clostridia vaccines for cattle, including those

for the development of new immunogens. In the last few years, new adjuvants and the use of mutant or recombinant antigens in the formulation of clostridial vaccines were tested, showing promising results. On the other hand, these studies focused only on the official tests and/or peak of antibodies in target species, which, according to the present study, does not guarantee a one-year long lasting humoral response in cattle. The present study showed that the humoral response induced by approved commercial clostridia vaccines reduced to undetectable levels before 240 days, which may increase the susceptibility of the animals to these diseases until the recommended period of revaccination.

Conflict of interest: No

P 8

The requirement for co-germinants during *C. difficile* spore germination is influenced by mutations in *yabG* and *cspA*

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C. difficile spores germinate in response to cholic acid derivatives, such as taurocholate (TA), and amino acids, such as glycine or alanine. Although the receptor with which bile acids are recognized (germinant receptor) is known, the amino acid co-germinant receptor has remained elusive. Here, we used EMS mutagenesis to generate mutants with altered requirements for the amino acid co-germinant, similar to the strategy we used previously to identify the bile acid germinant receptor, CspC. Surprisingly, we identified strains that do not require co-germinants, and the mutant spores germinated in response to TA alone. Upon sequencing these mutants, we identified different mutations in *yabG*. In *C. difficile*, *yabG* expression is required for the processing of key germination components to their mature forms (e.g., CspBA to CspB and CspA). A defined *yabG* mutant exacerbated the EMS mutant phenotype. Building upon this work, we found that small deletions in cspA resulted in spores that germinated in the presence of TA alone without the requirement of a co-germinant. *cspA* encodes a pseudoprotease that was previously shown to be important for incorporation of the CspC germinant receptor. Herein, our study builds upon the role of CspA during *C. difficile* spore germination by providing evidence that CspA is important for recognition of co-germinants during *C. difficile* spore germination. Our work suggests that two pseudoproteases (CspC and CspA) likely function as the *C. difficile* germinant receptors.

Conflict of interest: No

P 9

Loop-mediated isothermal amplification combined with a lateral flow biosensor for detection of *Clostridium perfringens*

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Clostridium perfringens is an important foodborne pathogen causing food poisoning. Definitive detection by standard culture method is time-consuming and labor intensive, therefore, it is necessary to develop a method for rapid and sensitive point-of-care detection. Here, loop-mediated isothermal amplification (LAMP) combined with a chromatographic lateral-flow biosensor (LFB) was developed for rapid and visual detection of cpa-specific *C. perfringens* amplicon. The specificity and sensitivity of LAMP-LFB were compared to conventional PCR method. A total of 30 bacterial strains including 5 *C. perfringens* and 25 other strains were tested in this study. No false positive amplification was observed, indicating 100% specificity. The sensitivity of the LAMP-LFB and PCR assays were 10 and 10^4 CFU/ml, respectively in culture using boiling method for genomic DNA extraction. In artificially inoculated food sample, the sensitivity of LAMP-LFB and PCR were 10^3 CFU/ml and 10^4 CFU/ml respectively without enrichment. However, with the enrichment, the sensitivity of both LAMP-LFB and PCR were 1 CFU/ml. In conclusion, LAMP-LFB is easy, simple and powerful tool for the detection of *C. perfringens*.

Conflict of interest: No

P 10

In silico analysis of ATP-binding cassette transporter gene family in *Clostridium difficile* 630

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The ATP-binding cassette (ABC) transporters represent one of the largest transporter gene families in all organisms. They function in substrate translocation across biological membrane using energy from ATP hydrolysis. Although the growing numbers of identified ABC transporters are involved in drug resistance, the knowledge of ABC systems in *C. difficile* are still inadequate. In this study, all putative ABC proteins encoded by *C. difficile* 630 genome were identified and categorized using computer-aided method. A total of 230 putative ABC genes were preliminary identified and their corresponding proteins account for a significant 6.12 % of total chromosomal proteins. Based on our analysis, they could be classified into 11 different subfamilies with the biological roles on nutrients uptake, compound extrusion and non-transport activity. Interestingly, the ABC drug efflux pumps occupied one third of predicted complete systems, rendering the ability of *C. difficile* to adapt and resist diverse antibiotic conditions. Furthermore, transcriptomic and proteomic data in *C. difficile* were overlaid to elucidate the significance of ABC transporters on antibiotic resistance. The in-depth investigation of the whole set of *C. difficile* ABC systems and their phylogenetic relationship will provide a comprehensive information on their biological role associated with the virulence, which could be useful for therapeutics development to reduce antibiotic resistance in this organism.

Conflict of interest: No

P 11

Characters of mobile genetic elements of *Clostridium difficile* through whole genome sequencing

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Horizontal gene transfer of mobile genetic elements (MGEs) contribute to the mosaic genome of *Clostridium difficile*, resulting in acquisition of new phenotypes, including drug resistance and reconstruction of the genomes [1,2]. In this study, we focus on analyzing the transposons, prophage, CRISPR array, and plasmids according to the second-, third-generation whole genome sequencing (WGS) of clade 4 and 5 *C. difficile* isolates, respectively. The PaLoc and CdtLoc regions among the three isolates in clade 5 were almost identical. Specifically, the location and length of deletions and insertions (indels) were the same, except the 661 bp deletion within *tcdA*, which was present only in isolate 10010 and 12038. Importantly, a point mutation at position 184 and Δ39-bp deletion within *tcdC* was detected in all three ST 11 *C. difficile* isolates.
Tn*4453a/b* carrying aac (6')aph (2'') but not catD was found in both clade 4 and clade 5 isolates (RT078). The number and type of transposons for clade 4 and 5 were a little bit different, for example, CTn*1*, CTn*4*, and CTn*6* were only identified in isolates from clade 5. Isolates from clade 4 were estimated to have more prophages than that in clade 5. In this study, the CRISPR arrays reached 12.5 and 13 arrays/genome in clade 4 and clade 5, respectively, which was significantly higher than previous report (8.5 arrays/genome) [3]. Importantly, RT078 display unique spacers compared with other two new RTs from clade5. Due to the third-generation WGS, two plasmids of two isolates in clade 5 were confirmed. In clade4, the rate of multidrug resistant rate (MDR), reached as high as 97.30%, much higher than that in previous report [4,5], while in clade 5, there was only one isolate resistant to on antibiotic.
In conclusion, we analyzed HGT related elements involved in shaping the genome and biological features including antibiotic susceptibility phenotype of *C. difficile* in China. Our findings provide important new insights into the mechanism of genome remodeling of *C. difficile*, and offer a new method for typing and tracing the origins of closely- related isolates.

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Conflict of interest: No

P 12/FL 2

Exploring Oligo-MM mice to study precision microbiome reconstitution against *Clostridioides difficile* infections

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Clostridioides difficile is gram-positive, spore forming bacteria, which causes severe diarrhea and life-threatening complications in infected individuals. In many developed countries, *C. difficile* infection (CDI) is now the major cause of antibiotic-associated nosocomial infections. The standard treatment for CDI are antibiotics, however, up to 30% of patients are suffering from recurrent CDI. Transfer of fecal material from healthy individuals is able to cure CDI, demonstrating that the microbiota serves an important role in preventing CDI. Recent studies have demonstrated that conversion of primary to secondary bile acids (BA) by specific commensals is important for this colonization resistance (CR). Moreover, restoration of secondary BA production by microbiota engineering is sufficient to reduce susceptibility to CDI in mice. However, restoration of secondary BA may not be sufficient to fully recover CR1.
We hypothesize that the presence of secondary BA producers (secBAP) is not sufficient to completely restore CR against *C. difficile* and want to explore gnotobiotic mouse models to investigate the interplay of secBAP with other commensals. Specifically, we aim to identify bacteria that cooperate with secBAP to inhibit the germination and growth of *C. difficile*.
We are using a gnotobiotic mouse model harboring twelve mouse-microbiota derived bacteria (OMM), but lacking bacteria that produce secondary BA.2,3 Additionally evaluated commensals are derived from the MiBC and our internal strain collection. OMM mice were infected with *C. difficile* (VPI 10463). CDI was monitored by following weight loss, colonization assays and ELISA for inflammatory markers and toxin production. Also, secondary BA were measured.
OMM mice infected with *C. difficile* do not show any CR, even without antibiotic pretreatment. To examine the effect of specific bacteria on the CDI, the OMM mice were in addition colonized: group 1 received secBAP, group 2 was colonized by a consortium of eight bacteria without secBAP, and group 3 received both. Upon infection, the mice from group 3 showed the highest protection against CDI 3

d.p.i. as demonstrated by reduced colonization and no body weight loss. While group 1 did have a better protection than the OMM, it was still higher colonized with *C. difficile* than group 3 already at early time points. The protection in group 1 was further decreased 3 d.p.i. The amounts of primary and secondary BAs were significantly higher in group 3 compared with OMM mice and group 1 and 2. This suggests that supplementing secBAP with additional commensals results in improved and extended protection against CDI caused by a higher amount of secondary BA.
We implemented a gnobiotic mouse model that will aid in the search for bacteria that confer a heightened CR against CDI. Even though secBAP were shown in our study to provide partial protection similar to what other (pre)clinical studies have demonstrated, their sole transfer does not provide an extended protection against CDI. This leads to our working hypothesis that secBAP need other bacteria to keep their colonization stable in low-grade inflammation.

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Conflict of interest: No

P 13

Peptidyl-prolyl-cis/trans-isomerases contribute to virulence and key metabolic processes in *Clostridium (Clostridioides) difficile*

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Bacterial peptidyl-prolyl-cis/trans-isomerases (PPIases) constitute a highly conserved protein family, members of which commonly accelarate protein folding. As such they are key to protein maturation, stability and dynamics. Here, we discuss bacterial PPIases as relevant modulators of virulence, and their implications on *Clostridium* physiology on the basis of our findigs on two representatives of this family in *C. difficile*, PrsA2 and PpiB. The membrane bound PrsA2 is a classical parvulin-type PPIase with a preference for positively charged amino acids preceding the proline residue in its substrates. Its inactivation using ClosTron reduces the germination efficiency in response to taurocholic acid, and increases resistance to the secondary bile acids lithocholic and deoxycholic acid. Furthermore, the absence of PrsA2 has significant impact on the colonization of mice by *C. difficile* 630. In case of the cyclophilin-type PPIase, PpiB, we present the first interactomic network of this protein in this pathogen. Among the diverse interaction partners of PpiB are major enzymes of the amino acid-dependent energy and the glucose-derived central metabolism as well as proteins of the general, oxidative, alkaline and cold shock response and regulators of transcription, translation and protein folding. Most interestingly, PpiB interacts functionally with CysK, a crucial enzyme of the cysteine-metabolism, which is in accordance with the decreased cysteine-tolerance and increased toxin levels of the *ΔppiB* mutant. Accordingly, we propose that PPIases in Clostiridium difficile contribute to its pathogenicity with implications for alternative therapeutic strategies.

Conflict of interest: No

P 14

In vitro evolution leads to a compensation of the reduced fitness associated with Fidaxomicin resistance in *C. difficile*

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Fidaxomicin (FDX) is an approved drug for *C. difficile* therapy, which targets the RNA polymerase (*rpoB*). Resistance development to Fidaxomicin is so far uncommon in clinical isolates, although strains with lower fidaxomicin susceptibility can be easily generated *in vitro* by exchanging the valine at position 1143 of RpoB (e.g. V1143D, V1143G, V1143F) [1] [2]. These *rpoB* mutations were associated with a substantial growth defect and this reduced fitness might account for the low frequency with which these strains were isolated from clinical specimens. However, we recently identified and described the first pathogenic clinical *C. difficile* isolate (DSM 105001) with markedly reduced fidaxomicin susceptibility (MIC >64 mg/L). [3]. This strain carries the V1143D mutation in RpoB, but surprisingly possesses an unaltered growth rate. We speculated that this might be due to compensatory mechanisms that take place during in vivo selection of mutants. To further investigate this hypothesis, we used the *C. difficile* 630Δ*erm rpoBV1143D* mutant, which shows the expected reduced growth rate, for *in vitro* evolution experiments. We passaged *C. difficile* 630Δ*erm rpoBV1143D* daily in the presence of sub-lethal FDX concentrations. After 20 days, we could observe a marked increase in growth rate of the population in comparison to the progenitor strain. Individual clones obtained from this population also showed the increased growth rate. We are now in the process to apply whole genome sequencing on individual clones in order to identify putative point mutations that can compensate the *rpoBV1143D* mediated growth reduction.

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Conflict of interest: No

P 15

Elucidating the oxidative stress response of *Clostridioides difficile*

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Clostridioides difficile 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 highly strain-dependent oxygen tolerance of the pathogen [1].
Screening of the *C. difficile* 630Δ*erm* genome revealed candidates putatively involved in the oxidative stress response and described in other bacteria to mediate tolerance against oxidative stress. These may explain the high tolerance of *C. difficile* to oxidative conditions.
In particular, a ruberythrin, encoded by rbr1, is highly expressed in *C. difficile* 630Δ*erm* according to proteomics studies. The gene is located in an operon with genes encoding the transcriptional repressor PerR; a desulfoferrodoxin and a glutamate dehydrogenase with a rubredoxin like structure at the N-terminus. All three proteins are also highly abundant in the late exponential phase of growth in *C. difficile* 630Δ*erm*. We use CRISPR-Cas genome editing to generate knock-out mutants of the genes encoded in the perR operon. Moreover, we screened the DNA 100 bp upstream of the coding region of all putative oxidative stress genes for possible PerR-binding boxes.
Furthermore, we focused our investigation on flavodoxins – small proteins featuring a flavin mononucleotide cofactor. An involvement of flavodoxins in the oxidative stress response and their vital role as electron donors in essential metabolic processes has been reported [2]. The genome of *C. difficile* encodes seven highly diverse flavodoxins. We used Northern blot analysis for profiling the expression of the flavodoxins under different conditions.
C. 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 the detoxification of reactive oxygen species. An abrogation of oxidative adaptation mechanisms could be a starting point for the development of novel treatment strategies.

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Conflict of interest: No

P 16

Superlative activity of teicoplanin on vegetative cells and spore outgrowth in *Clostridium difficile*

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An increased incidence of *Clostridium difficile* infection (CDI) in community and healthcare settings due to emergence of new strains, ability of *C. difficile* to form spore, and its resistance to commonly used antibiotics has triggered an attempt to look for alternative treatment options. In this study, we demonstrated an antimicrobial activity of teicoplanin in comparison to vancomycin on 30 distinct *C. difficile* isolates from different host origins, with minimal inhibitory concentration (MIC) ranging from 0.03 - 0.25 µg/mL. We also examined the efficacy of teicoplanin; and its comparator, for its ability to block *C. difficile* spore germination and outgrowth. Exposure of *C. difficile* spores to broth supplemented with varying concentrations of antimicrobial agents did not affect the initiation of germination ($P > 0.05$), but all inhibited further outgrowth to vegetative cells ($P < 0.0001$). These findings were well-supported by differences in acquisition of stain affinity to germinating cells and production of an aberrant bacterial morphology following antibiotic therapy when examined by light microscopy. Altogether, teicoplanin has proven superlative to its close relative vancomycin and remains valid for clinical use in Thailand, but an attempt to find an inhibitor for spore germination is further pursued.

Conflict of interest: No

P 17

Environmentally-regulated cell fate shapes biofilm of *Clostridium perfringens*

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Most microbes live in microbial communities called biofilm in natural environments. Cells in biofilms are embedded in self-produced extracellular polymeric substances (EPS). Biofilms show increased tolerance to environmental stresses and thus are widely recognized as causes of infectious diseases and environmental contaminants. *Clostridium perfringens* is a causative agent of gas gangrene and food poisoning. We found that *C. perfringens* forms biofilms with a different structure in response to temperature (1). At 37°C, cells adhere densely to surfaces, forming thin biofilms. In contrast, at 25°C cells produce a threadlike EPS that facilitates the formation of a thick, elastic, pellicle-like biofilm. Temperature is an environmental cue that alters between the outside and inside of the host, which suggests that the morphological changes of biofilms are involved in the pathogenesis of *C. perfringens*.
Here, we identified the gene responsible for production of the threadlike extracellular EPS that is indispensable for pellicle-like biofilm formation. We constructed anaerobic fluorescent protein reporter strains to detect the ESP gene expression at a single-cell level in *C. perfringens*. Fluorescent reporter analysis reveals that there are two types of cells within the overall population. In one type, the EPS gene promoter is activated. In the other type, the EPS gene promoter is repressed, and the cells attach to the surface. This heterogeneity is further controlled by temperature, which greatly influences biofilm morphology. Futhermore, we observed that cells expressing EPS overlie the EPS-repressed cells which are located near the surface, by confocal laser scanning microscopy. The deletion of the gene necessary for type IV pili formation, which are involved in surface attachment of *C. perfringens*, activates the EPS gene expression in the whole population through the positive feedback regulation via a two-component system.
Our data demonstrate heterogeneous expression of the gene responsible for the threadlike EPS production determines cell fate in *C. perfringens*; cells may either attach to surfaces through Type IV pili production or produce extracellular matrix. We believe that heterogeneity of EPS gene expression regulated through

surrounding temperature is a strategy for adaptation to the inter-host and external environments, which modulates morphological changes of biofilm architecture.

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Conflict of interest: No

P 18

Identification of an Important Orphan Histidine Kinase for Initiating Sporulation and Enterotoxin Production by *Clostridium perfringens* Type F Strain SM101

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Clostridium perfringens type F strains cause the 2nd most common bacterial foodborne illness and many cases of nonfoodborne human gastrointestinal diseases, including antibiotic-associated diarrhea. Sporulation plays two critical roles during type F enteric disease. First, it produces highly resistant spores that facilitate type F strain survival in the food and nosocomial environments. Second, production of *C. perfringens* enterotoxin (CPE), the toxin responsible for causing the enteric symptoms of type F diseases, only occurs in sporulating cells. While later steps in *C. perfringens* sporulation have been determined, the process leading to phosphorylation of Spo0A, the master early regulator of sporulation and consequent CPE production, has been unknown. Using Targetron mutagenesis, the current study identified the orphan histidine kinase CPR0195 as an important factor regulating *C. perfringens* sporulation and CPE production. Specifically, a CPR0195 null mutant of type F strain SM101 made 103-fold fewer spores than its wild-type parent and produced no detectable CPE. In contrast, a null mutant of another putative *C. perfringens* orphan histidine kinase (CPR1055) did not significantly affect sporulation or CPE production. Studies using a spoIIA operon promoter-driven reporter plasmid indicated that CPR0195 functions early during sporulation, i.e., prior to production of sporulation-associated sigma factors. Furthermore, in vitro studies showed the CPR0195 kinase domain can autophosphorylate and phosphorylate Spo0A. These results support CPR0195 as an important kinase that initiates *C. perfringens* sporulation by directly phosphorylating Spo0A. This kinase could represent a novel therapeutic target to block *C. perfringens* sporulation and CPE production during type F disease.

Conflict of interest: No

P 19

Sporicidal and antibacterial dual efficacy of vancomycin-conjugated nanoparticles against *Clostridium difficile* infection

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Current antibiotic treatments failing to eliminate *Clostridium difficile* (*C. difficile*) spores and causing refractory *C. difficile* infection raise an unmet need for a spore-targeting antimicrobial treatment with minimal side effects. We developed a sporicidal and antimicrobial vancomycin-loaded spore-targeting iron oxide nanoparticle (van-IONP) that selectively binds to *C. difficile* spores. Cryo-electron microscopy showed that vancomycin-loaded nanoparticles can target and completely cover spore surfaces. It not only successfully delayed the germination of the spores at initial but inhibit ~50% of newly germinated vegetative cells after 48 hours of incubation. The nanoparticles also inhibited interaction of the spore with HT-29 intestinal mucosal cells in vitro. In a *C. difficile* infected mouse model, the van-IONP significantly protected the mice from infected by *C. difficile* infection, reducing intestinal inflammation, and facilitated superior mucosal viability compared with equal doses of free vancomycin. This dual-function targeted delivery therapy showed advantages over traditional therapeutics in treating *C. difficile* infection.

Conflict of interest: No

P 20

Longitudinal incidence of *Clostridioides difficile* infection (CDI) in the US Medicare 5% Random Sample

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Most studies of CDI incidence are limited to one year of data to allow for development of CDI and/or investigate annual CDI incidence. Data are limited on an individual's "lifetime" incidence of CDI. How "lifetime" incidence differs from 1 year incidence is not known.

Medicare 5% random sample claims data from 1999 through 2015 were utilized. Age at time of entry into the data were split into 5 year increments: 65-69, 70-74, etc. The date of CDI onset was defined as the first date coded for CDI (008.45). To avoid misclassification of recurrent CDI as an incident case, all persons had to have at least 84 consecutive days without coding for CDI after entry into the database. People who were coded for CDI were eligible for another incident episode of CDI if there was at least an 84 day period without coding for CDI. The number of individuals with at least one episode of CDI was determined, as was time to first CDI episode and total number of CDI episodes. CDI incidence rates using all person years of observation versus 1 year of observation were calculated. Chi-square tests were used to compare lifetime versus 1 year CDI incidence rates.

There were 3,289,475 people included with 9,525 CDI episodes and 3,151,844 person years of observation during the first year after entry into the data, and 149,116 CDI episodes and 24,448,106 person years of lifetime observation. There were 128,294 (3.9%) people who had at least one episode of CDI. Overall, the median time to first CDI episode was 94 months. The overall lifetime CDI incidence rate (610 / 100,000 person-years) was significantly higher than the overall 1 year rate (302 / 100,000 person-years) and rates for all age groups ($P<0.0001$), except for the ≥95 age group. People had significantly more comorbidities, acute conditions, frailty indicators, infections, and healthcare exposures in the 84 days prior to first episode of CDI versus the first 84 days in the database.

The median time to first CDI episode was >7 years after entry into the Medicare database, and the CDI incidence rate was significantly higher when all years of data were used to identify CDI versus only 1 year of claims data. People were much sicker at the time of CDI than when first entering the database, Limiting CDI incidence analyses to 1 year of data significantly underestimates an individual's lifetime risk for CDI.

Conflict of interest: Yes

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P 21

Direct inhibition of toxin action reveals a new role for bile acids in modulating *Clostridioides difficile* pathogenesis

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Clostridioides difficile is an anaerobic, spore-forming bacterium that can, in permissive conditions, germinate and secrete toxins that damage the intestinal epithelium leading to diarrhea and colitis. While it is known that disrupting the healthy gut microbiota with antibiotics is an essential trigger for this process, the precise factors that enable *C. difficile* disease pathogenesis and ultimately determine disease susceptibility are unknown. Here, we show that bile acids, which play a well-established role in *C. difficile* germination and outgrowth, also directly bind and inhibit TcdB toxin, the primary virulence determinant of *C. difficile*. We show that binding and inhibition of TcdB is dependent on the C-terminal combined repetitive oligopeptide (CROP) domain. Further, we show that bile acid binding induces a major structural change in toxin structure that stabilizes toxin structure and prevents target-cell binding. In addition to highlighting a new role for bile acids in conferring colonization resistance against *C. difficile*, these findings uncover a new mechanistic approach to inhibit toxin pathogenesis and treat *C. difficile* disease.

Conflict of interest: No

P 22

Increasing prevalence of *Clostridioides difficile* REA Group DH (RT106) associated with cefepime exposure

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Cephalosporins have been associated with *Clostridioides difficile* infection (CDI), although the risk may be different for individual cephalosporins. There are marked differences in pharmacokinetic profiles and gut concentrations among different cephalosporins even within the same generation or class. One cephalosporin that has been used more frequently in the U.S. over the past 10 years is cefepime, a 4th generation cephalosporin that is renally excreted, but achieves significant concentrations in the gall bladder and bile. Use of cefepime in patients with febrile neutropenia has been associated with increased the rates of CDI when compared to meropenem. There has also been a change in the epidemiology of CDI in the U.S. over the last 5-10 years, with decreased prevalence of the restriction endonuclease analysis (REA) group BI (RT027) and increased prevalence of other strains, including REA group DH (RT106).
From 2013 to 2015, we studied 223 patients from the Hines VA Hospital with first CDI episodes. REA and antibiotic susceptibility testing were performed on the recovered *C. difficile* isolates, and the electronic medical record was reviewed to determine antibiotic exposures 6 weeks prior to developing CDI. Antibiotic susceptibilities were determined by E-test for ceftriaxone and cefepime on 14 representative DH isolates.
Among the 223 patients, 27 were infected with REA group DH (12%), and 42 (19%) and 35 (16%) were infected with REA groups BI and Y, respectively. Third- and fourth-generation cephalosporin exposure was documented in 14/27 (52%) DH-infected patients compared to 11/42 (26%) and 5/35 (14%) of BI- and Y-infected patients, respectively (P=0.03 for DH compared to BI and 0.001 for DH compared to Y). Cefepime exposure was documented in 7/27 (26%) DH-infected patients compared to 3/42 (7%) and 2/35 (6%) of BI- and Y-infected patients, respectively (P=0.03 for DH compared to BI and 0.03 for DH compared to Y). All DH isolates were highly resistant to cefepime (MIC>256 µg/mL) but variably resistant to ceftriaxone (MIC range 16 to >256 µg/mL). Whole genome sequencing

was performed on a clonal group of DH isolates from 2 pediatric patients from a nearby free-standing children's hospital in which antibiotic resistance to multiple antibiotics developed after prolonged hospitalization and antibiotic exposure. Increased MICs to ceftriaxone (from 48 to 96-128 µg/mL) was associated with acquisition of a missense mutation in a penicillin-binding protein gene (G2834T, Ser945Ile). However, all isolates were fully resistant to cefepime (>256 µg/mL).
We noted an association between cefepime use and CDI with REA group DH (RT106) among patients with first CDI episodes. Prevalence of REA group DH has increased significantly at our hospital since 2005-2007, when we noted a predominance of REA group BI and an association with fluoroquinolone use. The mechanism of resistance to cefepime in *C. difficile* and prevalence of high-level resistance in other *C. difficile* strains warrants further investigation.

Conflict of interest: No

P 23

Metabolic adaptation of *Clostridioides difficile*

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The obligate anaerobe, spore forming bacterium *Clostridioides difficile* (formerly *Clostridium difficile*) causes nosocomial and community acquired diarrhea often associated with antibiotic therapy. Major virulence factors of the bacterium are the two large clostridial toxins TcdA and TcdB. The production of both toxins was found strongly connected to the metabolism and the nutritional status of the growth environment. Following the growth course of *C. difficile*, the reductive equivalent consuming pathways used were sequentially shifted from proline via leucine/phenylalanine to the central carbon metabolism first to butanoate fermentation and then further to lactate fermentation. The toxin production was found correlated mainly to fluxes of the central carbon metabolism. Toxin formation in the supernatant was detected when the flux changed from butanoate to lactate synthesis in the late stationary phase.
Transcriptome and metabolome data obtained under defined growth conditions addressing different parts of the central carbon metabolism. Data were integrated to uncover multiple adaptation strategies of *C. difficile* to altered substrate availability, in this case glucose, lactate or cysteine supplementation, all being metabolized via pyruvate. Interestingly, lactate supplementation altered only a very limited number of genes and metabolites while cysteine and glucose showed a very broad impact on the metabolism of *C. difficile*. Cysteine supplementation was also associated with iron limitation due to the formation of iron sulphide. The integrated view derived from the combination of transcriptome and metabolome data allowed us to uncover the major metabolic strategies that are used by the clostridial cells to maintain its cellular homeostasis and ensure survival under starvation and stress conditions.

Conflict of interest: No

P 24

Distinct phenotypes of *Clostridioides difficile* 630 *agrB1* and *agrD1* mutants

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Clostridioides difficile accessory gene regulator-1 (*agr1*) locus encodes a membrane protease and an autoinducing peptide. In contrast to prototypical Agr systems, *C. difficile* agr1 lacks genes for a sensor histidine kinase and response regulator. How the Agr1 system functions in the absence of a cognate histidine kinase and response regulator is unknown. In this study, we generated individual knockouts of *agrB1* and *agrD1* in *C. difficile* 630. To make precise deletions in individual genes and to avoid polar effects we developed a CRISPR-Cas9 nickase genome editing system to generate *agrB1-/agrD1+*, *agrB1+/agrD1-* , and *agrB1-/agrD1- C. difficile* mutants. By analyzing these *agr* mutants, we identified phenotypes distinct to the *agrB1-/agrD1-* but not found in the agrB1-/agrD1+ and *agrB1+/agrD1-*. Conversely, we found phenotypes distinct to *agrB1-/agrD1+* and *agrB1+/agrD1-*, but not detected in *agrB1-/agrD1-*. *C. difficile agrB1-/agrD1-* showed reduced toxin production, flagella production, and attenuated motility. Unexpectedly, *agrB1-/ agrD1+* and *agrB1+/agrD1-* strains exhibited hyperexpression of TcdA, and a differential histidine protein phosphorylation profile, but no changes in motility. These findings suggest that the Agr1 system in *C. difficile* has multilayered regulation where individual Agr1 components are connected to other pathways to exert tight regulation of target genes.

Conflict of interest: No

P 25

Amino acids 1769-1787 contribute to structural integrity and restrict spontaneous autoprocessing in *Clostridioides difficile* TcdB2

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Clostridioides difficile TcdB is an intracellular toxin comprised of multiple functional domains which confer the cytotoxic activities of the protein [1]. Cellular intoxication involves conformational changes that must occur in a precise order for intoxication to be successful [2]. The goal of our work is to identify specific regions of TcdB involved in these conformational changes, and develop strategies for targeting these regions as an anti-toxin therapeutic. Previous work from our lab found that a synthetic peptide derived from TcdB2 amino acids 1769-1787 promotes conformational changes and inhibits TcdB cytotoxicity [3]. In this study, we used site-directed mutagenesis to generate a series of deletion mutants targeted within and proximal to TcdB2 residues 1769-1787, and found one in particular, TcdB2Δ1769-1787, which showed a complete loss of cytotoxic activity and did not bind to cells. TcdB2Δ1769-1787 also exhibited spontaneous autoprocessing in the absence of IP 6, indicating the mutant exists in a conformation that allows the active conformer of the autoprocessing domain to form. Despite the lack of detectable cell interaction and toxicity by TcdB2Δ1769-1787, vaccination with this deletion mutant provided protection from disease in a murine model of CDI. Mice vaccinated with TcdBΔ1769-1787 exhibited a protective IgG1 immune response, and produced antibodies capable of neutralizing full-length TcdB2, suggesting the mutant retained critical neutralizing epitopes. Collectively, these findings indicate TcdB2Δ1769-1787 is a promising vaccine candidate, and that therapies which destabilize the 1769-1787 region might be used to inactivate this toxin during the course of disease.

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Conflict of interest: No

P 26

Regulation of *Clostridioides difficile* sporulation and toxin production by the multifunctional protein, RstA

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The anaerobic spore-former, *Clostridioides difficile*, causes significant diarrheal disease in humans and other mammals. Infection begins with the ingestion of dormant spores present in the environment, which subsequently germinate within the host gastrointestinal tract. Here, the vegetative cells proliferate and secrete two exotoxins, TcdA and TcdB, which cause the symptoms of the disease. Although spore formation and toxin production are critical for *C. difficile* pathogenesis, the regulatory links between these two physiological processes are not well understood and are strain-dependent. Previously, we identified a *C. difficile* regulator, RstA, that promotes sporulation initiation through an unknown mechanism and directly and indirectly represses toxin gene transcription. In this study, we investigate the function of RstA by complementation analyses using both alanine-scanning site-directed mutagenesis on conserved RstA residues and multi-species RstA orthologs and chimeras. We discovered one amino acid mutation that resulted in a hypersporulation phenotype, yet had no effect on TcdA levels. Additionally, mutations within the C-terminal domains of RstA uncovered several amino acids that are important for toxin production, supporting the hypothesis that the C-terminal domains of RstA control its N-terminal DNA-binding activity. Complementation analyses with other clostridial RstA orthologs revealed that the *Clostridium* sordelli RstA ortholog restored sporulation, but not toxin production, while the *Clostridium acetobutylicum* RstA ortholog conferred the opposite effect, revealing conserved regions and residues necessary for RstA function in *C. difficile*. Additional in vivo and in vitro studies with these RstA site-directed mutants and orthologs will further elucidate RstA function and the molecular mechanisms by which RstA positively controls sporulation. Finally, analysis of RstA-dependent control of toxin gene expression in three *C. difficile* strains reveal differences in toxin production regulation between motile and non-motile strains. Altogether, our data indicate that RstA coordinates *C. difficile* sporulation and toxin production regulation through separate domains and through mechanisms that are evolutionarily-conserved among clostridia and that the regulatory pathways by which RstA controls toxin production are strain-dependent.

Conflict of interest: No

P 27

Evaluation of the VirS membrane protein as a signaling peptide receptor for the *C. perfringens* Agr-like quorum sensing system

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Previous studies showed that the Agr-like quorum sensing (QS) system of *Clostridium perfringens* controls production of many toxins, including beta toxin (CPB) (1, 2). The Agr-like QS becomes activated when 5R, a 5-mer cyclic signaling peptide (SP), binds to an unidentified receptor. Since CPB production is positively regulated by both Agr-like QS and VirR/S two component regulatory system, the VirS membrane protein has been suggested to be SP receptor. To begin evaluating whether VirS is a/the SP receptor for the *C. perfringens* Agr-like QS system, the *virS* genes in strains CN3685 and CN1795 were inactivated by Targetron mutagenesis and those mutants were validated by PCR, RT-PCR and Southern blotting. The peptide Biotin-5R, corresponding to biotinylated SP, was added to two *C. perfringens* strains and their isogenic *virS* mutants; after washing, a streptavidin-AlexaFluor 488 conjugate was added, followed by washing and evaluating bound Biotin-5R by measuring fluorescence. Results obtained indicate that the *virS* null mutants bind less Biotin-5R than their isogenic parents, consistent with VirS being a/the SP receptor. To further evaluate the role of VirS as a SP receptor, we sequenced the *virS* genes of CN3685 and CN1795 to seek an explanation for why agrBD mutants of both *C. perfringens* strains respond to the natural 5-mer SP, but only the CN3685 agrBD mutant can respond to 8R, which is SP plus a 3 amino acid tail (2). This sequencing identified a 6 amino acid insert that is present in the VirS of CN3685, but not CN1795. Based upon the differences in SP-based peptide sensitivity between the two *C. perfringens* strains, we constructed *virS* and *agrB* double null mutants of each strain and then complemented those strains to swap which VirS protein they express. Western blotting for beta toxin production (which is controlled by both VirS/R and the Agr-like QS system) will be performed to evaluate whether the 5R SP or 8R SP can signal the CN1795 double mutant when it is complemented to express the virS gene of CN3685. We are also now working to use Biotin-5R pulling down VirS directly from one of these *C. perfringens* strains to further confirm VirS as an Agr-like QS SP receptor.

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Conflict of interest: No

P 28

Effect of broad vs. narrow spectrum *Clostridiodes difficile* treatment on human stool bile acid composition over time

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Secondary bile acid production by a diverse commensal flora is thought to be a critical factor in preventing recurrence of *Clostridiodes difficile* infection (CDI). Key enzymes involved in this process are the bacterial-encoded bile salt hydrolases, and genes in the bile acid inducible (bai) operon critical for secondary bile acid synthesis. Ridinilazole is a novel narrow spectrum anti-*C. difficile* agent that demonstrated superior sustained clinical response compared to vancomycin in a Phase 2 clinical trial. Longitudinal sampling of subjects during this trial has allowed for assessment of metabolites differentially present in stools during and after therapy with a broad spectrum vs. narrow spectrum agent for CDI. Previous work to characterize the fecal microbiota over the course of this trial has shown that unlike vancomycin, ridinilazole has little effect on the commensal flora. We hypothesized that ridinilazole's microbiota-preserving effect is associated with lack of accumulation of conjugated primary bile acids and/or reaccumulation or persistence of secondary bile acids over the course of CDI treatment in ridinilazole-treated subjects, compared with vancomycin-treated subjects.

Aims: To compare in humans the effects of ridinilazole and vancomycin on bile acid profiles at various time points before and after initiation of treatment for CDI, and assess for correlations between bile acid profiles and predicted bacterial bile salt hydrolase gene abundances.

Sequential stool samples from a Phase 2 trial were obtained from subjects treated with either ridinilazole or vancomycin, ranging in times from CDI diagnosis, at end-of-therapy, and up to 40 days after diagnosis. Bile acids were measured by liquid chromatography-mass spectrometry (LC-MS). Using the previously determined microbiota profiles, metagenomic predictions of bile salt hydrolase gene abundances were performed using the PICRUSt algorithm. Possible relationships between these data and time/treatment group were then explored.

Stool bile acid compositions were different between ridinilazole-treated and

vancomycin-treated patients at end-of-therapy. Stool bile acid composition in vancomycin-treated subjects was dominated by conjugated primary bile acids, with decreased levels of secondary bile acids. Using a random forest classification model, at end-of-therapy, the ratio of stool conjugated bile acids to secondary bile acids was a significant predictor of treatment arm. At end-of-therapy the predicted bile salt hydrolase levels in vancomycin-treated subjects were low compared to ridinilazole-treated subjects. Furthermore, the ratio of stool conjugated bile acids to secondary bile acids was significantly associated with predicted abundances of bacterial bile salt hydrolase genes in ridinilazole-treated patients. Future work will focus on exploring correlations between specific taxa and bile acid composition, whether bile acid features at end-of-therapy are predictive of recurrence, and assessment of other metabolites that may be differentially present between times/treatments.

Conflict of interest: Yes

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P 29

Analysis of *Clostridium difficile* spore germination in fluid from the upper gastrointestinal tract of healthy adults

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Following perturbations in the gut microbiota, *Clostridium difficile* can colonize the gut and cause diarrhea and colitis. The germination of *C. difficile* spores on exposure to bile salts and co-germinants precedes disease. However, the impact of these germinants on the spore to vegetative cell transition in the human gastrointestinal (GI) tract are poorly understood, particularly in the upper GI tract. The primary objective of this study was to identify characteristics of the human upper GI tract that modulate *C. difficile* spore germination.
We employed a multilumen tube introduced into the human gastrointestinal tract via the oral cavity to sample intestinal fluid from the stomach, duodenum and jejunum of healthy individuals. Fluid was aseptically collected and stored in consideration of low sample biomass for 16S microbial community analysis. Bile salts were analyzed by LC-MS/MS. *C. difficile* spore germination was tested through ex vivo assays in fluid buffered at pH 7.
This study included 28 individuals (Sex: 19 M, 9 F; Age: 30.1 ±9.6; BMI: 25.9 ±4.4). Fluid pH was lowest in the duodenum (5.3 ±1.5) and highest in the mid jejunum (5.7 ±0.7). Ex vivo germination assays conducted at pH 7 demonstrated that fluid from all sections of the small intestine supported *C. difficile* germination with 50% of spores germinating in the duodenum and 99.7% germinating in the proximal jejunum. Logistic regression showed that location is a significant predictor of germination ($p < 0.05$) with higher efficiency in the proximal and mid jejunum than the duodenum and stomach. Taurocholate concentration was highest in the mid jejunum (1.1×10^3 µg/mL) and lowest in the duodenum (2.1×10^2 µg/mL); it also significantly correlated with germination.
Although germination occurred in fluid from all regions of the healthy human small intestine, germination efficiency varied across those regions. Not surprisingly, germination was associated with bile salt concentrations in those environments.

Our data indicates that little bile salt deconjugation occurs over the course of the human duodenum and jejunum. While current treatments for *C. difficile* infection focus on the vegetative state, future research may lead to treatments that either directly inhibit spore germination or alter the intestinal environment to limit it.

Conflict of interest: No

P 31

An infant botulism case associated with *Clostridium botulinum* strains in China

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Clostridium botulinum is a Gram-positive, anaerobic, sporeforming, rod-shaped bacterium. *C. botulinum* can produce the botulinum neurotoxins (BoNTs) responsible for causing botulism, which is a life-threatening disease in humans and animals. Botulism commonly results from either ingestion of preformed foodborne BoNT or from growth of *C. botulinum* in either the intestinal tract or wounds. In infants, botulism is caused by in vivo production of BoNT by germinating *C. botulinum* spores in the intestinal tract. Between 70 and 100 cases of infant botulism are reported in the United States annually. Infant botulism was rarely reported in China. The second reported event of the disease including three cases occurred in 2015. In the present study, one (the third case) of the three cases was identified and investigated to trace the sources of transmission. Samples from feces and foodstuffs were used to isolate *Clostridium botulinum* (*C. botulinum*) strains. Each isolate was obtained from the baby's feces and opened powdered infant rice cereal, respectively. In this case, the *C. botulinum* strains were identified and characterized by combined mouse bioassay, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and high-throughput sequencing including single nucleotide polymorphisms (SNP). Results showed that the disease was caused by a type B strain of *C. botulinum*. Strains associated with this case as well as isolates from stored and historical samples were phylogenetically analyzed and compared. *C. botulinum* type B isolates from the infant feces and from an opened container of infant rice cereal were indistinguishable, suggesting that opened container of infant rice cereal is likely to be the source of transmission of spores to the infant. It is not clear that how the opened container was contaminated and the child was exposed since environmental testing was not performed. However, botulism epidemics have a certain relationship with the geographical distribution of *C. botulinum* in China, and Hebei province is one district in which botulism frequently occurs. Botulism

in Hebei is caused mainly by *C. botulinum* types A and B, with the number of cases caused by type B exceeding that caused by type A. While the domain strain in Xinjiang province is type A which is different from other provinces. This study provides detailed information about usage of the three methods and references for dealing with other associated cases. In addition, three infant botulism cases identified in 2018 were also described here.

Conflict of interest: No

P 32

Risk factors for *Clostridium difficile* infection among hospital inpatients in Western Australia

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Clostridium difficile continues to pose a major public health threat in Australia, with high incidence and frequent community-based infections. Since 2010, mandatory reporting of cases of *C. difficile* infection (CDI) has been in place in all public hospitals in Western Australia (WA), which allows detailed investigation of the epidemiology of CDI to be performed. The aim of this study was to investigate risk factors for CDI among inpatients of public hospitals in Perth's North Metropolitan Health Service other than exposure to antimicrobials.
A case control study was performed, including all CDI cases aged ≥2 years recorded from July 2012-June 2014 who were admitted to three public hospitals (one 600-bed tertiary hospital, two 200-bed community acute hospitals). Corresponding hospital admission records in The Patient Administration System (PAS) were interrogated for details of admission and discharge status, care type, location prior to admission, principal diagnoses and comorbidities, and basic demographic details.
Cases were defined as having diarrhoea (stool assuming the shape of its container) and positive diagnostic PCR for *tcdB*. Controls were chosen by randomly selecting 3 patients admitted to the same study site on the same or following day as a CDI case. Univariate and multivariate odds ratios (ORs) for risk of outcome of CDI were calculated using logistic regression.
A total of 305 CDI cases and 915 controls were selected for analysis. The majority of admissions for CDI cases were non-elective (80.3%), and the care type was primarily acute care (91.8%) followed by rehabilitation (6.9%). Most patients were referred from home (78.4%) while 21.3% were transferred from other acute care hospitals, and 3.3% were admitted from residential aged care facilities (RACFs). Readmissions were 3.0%. The majority of cases were discharged home (71.8%) while 21.0% were transferred to other health care facilities, primarily other acute care hospitals or RACFs. Crude mortality was 7.2% (22 cases).
Following multivariate regression, age ≥65 y (OR 5.27, 95% CI 3.70-7.51), disease of the circulatory system (OR 2.28, 1.49-4.14) and inflammatory bowel disease

(OR 7.63, 1.59-36.59) were identified as risk factors for CDI. Elective admission (OR 0.15, 0.10-0.24), acute care (OR 0.10, 0.04-0.24) and comorbidity of infectious disease (excluding CDI, 0.06 (0.04-0.09) were negatively associated with outcome of CDI.

Among hospital inpatients in WA, CDI is most commonly associated with the known risk factor of advanced age. Non-elective and non-acute care patients are at increased risk of CDI. Inflammatory bowel disease patients are at particular risk of CDI, likely due to disrupted gut microbiota due to their illness. Infection control strategies should focus on these patient groups to try to reduce their risk of infection.

Conflict of interest: No

P 33

Phenotypic and genotypic analysis of *Clostridium difficile* strains isolated from children hospitalized in a large pediatric center in Poland- retrospective study

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Clostridium difficile infection (CDI) is found in children worldwide. Molecular characterization of *C. difficile* based on PCR-ribotyping and toxins genes detection is important for surveillance and infection control of CDI as well as antibiotic susceptibility testing. Limited data are available from children living in Poland. We present first time data of phenotypic and genotypic analysis of *C. difficile* strains isolated from children hospitalized in a large pediatric center between 2005 and 2006.
We report results of toxin profiles, PCR-ribotyping, and antibiotic susceptibility of thirty-seven *C. difficile* strains isolated form pediatric patients. All confirmed *C. difficile* isolates were characterized using capillary gel electrophoresis based ribotyping. Fragments of genes encoding TcdA, TcdB and CDT toxins were detected by PCR. Susceptibility to six antimicrobial drugs: metronidazole, vancomycin, ciprofloxacin, moxifloxacin, erythromycin and clindamycin was checked by the E-test.
We identified 18 PCR-ribotypes (RTs) of *C. difficile:* RT014 n=8; 21,6%, RT012 n=5; 13,5%, RT017 n=3; 8,1%, RT010 n=3; 8,1%, RT626 n=3; 8,1%, RT146 n=2; 5,4%, RT046 n=2; 5,4%, and one isolate of each RT: 001, 004, 005, 011, 018, 020, 029, 039, 054, 144, and 258. Most of them were toxigenic, A+B+CDT- was most prevalent type n=30 (81%), other isolates were non toxigenic n=7; 19%. All of *C. difficile* strains were susceptible to metronidazole and vancomycin, also when tested by the PCR for plasmid mediated metronidazole resistance. Among 37 tested strains all (100%) proved to be resistant to ciprofloxacin (MIC90 = 32mg/L), only one (2,7%) strain was resistant to moxifloxacin. The proportion of strains resistant to erythromycin was 37,8% (n=14), to clindamycin 35,1% (n=13). Our results showed diversity of *C. difficile*

strains of the studied pediatric hospital, but the frequently reported PCR-ribotype 265 in Dutch and Belgium children was not found. Limitation of this study is small number of tested isolates. Prospective study of *C. difficile* in pediatrics population is planned to better understanding epidemiology of CDI in this special population.

P 34

Impact of intestinal bile acids on germination and sporulation of clinical *Clostridiodes difficile* isolates

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With the emergence of virulent epidemic strains, *C. difficile* infection (CDI) has become the leading cause of hospital-acquired diarrhea. The disruption of the physiological gut microbiota by antibiotic intake is one of the major risk factors for CDI. After oral uptake of metabolic inactive spores, bile acids within the gut impact the germination and outgrowth of the bacterium. Previous studies on the effect of bile acids on *C. difficile* have shown that under standardized concentrations of 0.1 %, certain bile acids can inhibit the growth of *C. difficile*. The importance of the healthy gut microbiota to prevent CDI is well-known, assuming that gut microbiota prevent the outbreak of CDI by influencing bile acid-proportions within the intestine. However, intestinal bile acid concentrations differ from the standardized 0.1 % concentrations. In order to investigate the role of physiological bile acid concentrations and interstrain variability in the development of a CDI, we analyzed spore germination and growth-kinetics of 23 clinical isolates and tested the induction of sporulation for 8 clinical isolates under a) intestinal, b) fecal and, c) standardized 0.1 % bile acid concentrations. Usually inhibiting bile acids at intestinal concentrations did neither prevent spore germination nor outgrowth of *C. difficile* strains, suggesting that intestinal concentrations of inhibiting bile acids only have marginally effect on *C. difficile* spores. Analyzing the sporulation, intestinal concentrations of inhibiting bile acids had little influence on sporulation and only fecal concentrations inhibited vegetative growth and sporulation of *C. difficile*. These results indicate that intestinal inhibitory bile acid concentrations only might not be sufficient to restrain germination, outgrowth, or sporulation of *C. difficile*, whereas fecal concentrations can prevent growth and sporulation. The data highlight that further studies are needed to evaluate physiological relevant inhibitory conditions to prevent CDI.

Conflict of interest: No

P 35

Recombinant spores of *Bacillus subtilis* presenting fragment of *Clostridioides difficile* FliD protein fused with VQGEESNDK peptide

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The technology of heterologous proteins display on surface of *Bacillus subtilis* spores has been used in different applications since its invention almost 2 decades ago. *B. subtilis* spores have been used for presentation of enzymes, fluorescent proteins, peptides, and antigens (1). Two main approaches to spore surface display have been developed. First, the recombinant one, requires modification of *B. subtilis* genome to express a passenger protein in fusion with a spore coat protein enabling its incorporation into the forming spore coat. Second approach is based on the adsorption technique and enables presentation of native proteins on surface of spores produced by wild-type strains (2).

One of the most interesting applications of spores presenting heterologous proteins is the use as carriers of antigens in mucosal vaccines. Mucosal vaccines, despite a number of potential advantages over injectable ones (such as no need of injections and hence no risk of transmitting bloodborne diseases, and easy way of administration), are much less common. Most soluble protein antigens introduced via the mucosal route are poorly immunogenic and induce specific, long-lasting tolerance. Moreover, the problems with rapid antigen degradation on the mucosal surfaces and lack of appropriate mucosal adjuvants primarily contribute to their diminished usefulness. The technology of spore surface display seems to be a remedy for some of these drawbacks.

In this study, we have successfully designed and constructed recombinant spores displaying an antigen/adjuvant chimeric protein. We have used a fragment of *Clostridioides difficile* flagellar cap FliD protein as antigen and VQGEESNDK peptide, a fragment of human IL-1β, as adjuvant. Recombinant spores presenting FliD were able to elicit immune response in orally immunized mice which could be evaluated by detection of FliD-specific IgA antibodies in feces of immunized animals. Moreover, the presence of IL-1β fragment significantly changed characteristics of elicited immune response.

Obtained results show that recombinant spores presenting an antigen/adjuvant chimeric protein exhibit both properties in mucosal immunization of mice. Moreover, IL-1β fragment could serve as valuable adjuvant in *B. subtilis* spore-based mucosal vaccines.

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Conflict of interest: No

P 36

Constructing a crystal shell: the lattice of *Clostridium difficile* S-layer

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Clostridium difficile is the most frequent cause of nosocomial infection across Europe and US. This Gram-positive pathogen is able to form spores which survive in the environment, acting as transmission agent. Infections usually outbreaks as antibiotic-mediated diarrhoea and consequent dehydration but can evolve to severe health issues and cause death (Rupnik et al, 2009).
An important feature of the *C. difficile* cell is its surface layer (S-layer), the proteinaceous 2D-crystalline array that covers the bacterium cell. Primarily acting as a protective layer for the bacterium, it is also considered a molecular sieve but has been implicated in interactions with the host, including cell adhesion (Fagan & Fairweather, 2014). In *C. difficile*, the S-layer variability is related to antigenic variation, making it a promising, but so far unexploited, target for new therapeutics (Kirk et al, 2017).
The S-layer lattice is formed by the polymerisation of its main protein, S-layer protein A (SlpA). It is expressed as a pre-protein inside the cell which, once secreted, is processed into the mature SlpA, consisting of two S-layer proteins (SLP): the HMW and LMW SLP. They interact forming a tight complex, generating the paracrystalline S-layer arrangement. Nonetheless, it remains unclear how such a complex and array is formed.
Using X-ray crystallography, we have so far obtained structures of several SlpA variants, from different *C. difficile* strains. Despite the modest resolution (just under 3Å), these structures revealed key structural features necessary for the L/HMW complex as well as the main interactions involved in S-layer assembly. Our findings indicate that a mature SlpA complex is formed by an intricate assembly of α-helical and β-sheet sandwiches involving the interacting domains (LID and HID) from the two SLPs. These domains form an essential region not only for SlpA itself but also display key residues for SlpA-SlpA connection, designing the tiles

that form *C. difficile*'s S-layer. The S-layer assembly model was further verified by determining the structure of one SlpA variant where one of the LMW domains has been deleted.
This work represents the first structure of a complete S-layer protein in a human pathogen and contributes for a better understanding of S-layer assembly and organisation. Importantly, it also paves the way for identifying potential targets for *C. difficile* specific therapeutics targeting the S-layer.

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Conflict of interest: No

P 37

Investigating the biological contribution of *C. difficile* Type IV pili proteins

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Clostridium difficile has been identified as a multidrug resistant intestinal pathogen, which causes health concerns in hospital environments. Adhesion, colonisation and biofilm formation play important roles in *C. 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, membrane associated proteins, PilC, PilMN and PilO, 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 in the 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 individual TFP proteins in *C. difficile*. The effect on bacterial growth, motility, cell-surface attachment and biofilm formation abilities of deleting TFP genes in *C. difficile* was investigated. The focus has been genes on the main TFP locus, but the more distant minor pilins, PilJ and PilW, were also studied. Together, our results contribute to growing understanding how the different pilin subunits affect pili formation and provide new insights on the role of TFP in *C. difficile*.

Conflict of interest: No

P 38

Identification of phage-derived endolysins against *Clostridium difficile*

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In the light of worldwide increasing antibiotic resistant bacteria, phage endolysins which are peptidoglycan hydrolases have been suggested as promising antimicrobial agents. The aim of this study was to investigate endolysin from phages infecting *Clostridium difficile*, an important pathogen that causes enteric diseases including antibiotic-associated diarrhea and pseudomembranous colitis. The endolysin gene from four different *C. difficile* phages including ΦHN10, ΦHN16-1, ΦHN16-2, and ΦHN50 were identified. Bioinformatics analysis revealed that the endolysins from all these phages displayed 100% sequence identity. Endolysin sequence comparison revealed high conservation across endolysins from phages which infect *C. difficile*, implying functional relevance for *C. difficile* cell wall hydrolysis. Phage endolysins, designated CDhnLs, contain 271 amino acids with modular structure. It is composed of an N-terminal enzymatically active domain (EAD) and a C-terminal putative cell wall binding domain (CBD). The CDhnL was cloned, heterologously expressed and purified. Zymographic analysis showed that the hydrolytic activity of endolysin presented only in full-length and EAD but absent in CBD. This result confirmed catalytic function located at the N-terminus of the protein. Finally, cell lysis assay showed bactericidal activity of endolysin against *C. difficile* and the EAD exhibited higher lytic activity than those of the full-length protein. The findings of this study could be of importance to develop phage endolysin as antimicrobial agent against *C. difficile*.

Conflict of interest: No

P 39

Proteotyping of *Clostridioides difficile* as alternate Typing Method to Ribotyping is able to differentiate the Ribotype 027

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Clostridioides difficile, a Gram-positive spore-forming bacterium, is the leading cause of nosocomial diarrhea worldwide and therefore a substantial burden to the healthcare system. Over the past decade, the hypervirulent *C. difficile* PCR-ribotype (RT) 027 emerged rapidly all over the world what was associated with both, increased severity and mortality rates.
To prevent disease spread, it is of great importance to identify epidemic strains such as RT027 as fast as possible. Commonly used diagnostic methods, e.g. multi locus sequence typing (MLST) or PCR-ribotyping, are time-consuming. Therefore the aim of the study was to develop a proteotyping scheme using MALDI-TOF MS, a fast, inexpensive, and reliable method.
To establish a *C. difficile*-proteotyping scheme, we analyzed 1,462 *C. difficile* genomes and evaluated 109 ribotyped strains representative for five MLST clades (including 20 RT027 isolates) by mass spectrometry. MLST, based on whole genome sequences, and PCR-ribotyping were used as further reference methods. We were able to associate nine biomarkers with their encoding genes and include them in our *C. difficile*-proteotyping scheme. The most important finding was that one of the clades only contained isolates of the hypervirulent RT027.
In this study, proteotyping that was previously used for subtyping of several microbial species by our group, was now successfully established to subtype *C. difficile*. Using this technique, we were able to distinguish RT027 isolates from non-RT027 isolates what makes proteotyping a fast and valuable alternative to currently used diagnostic tools.

Conflict of interest: No

P 40

Characterizing human intestinal community for suppression of *Clostridioides difficile*

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Antibiotics disturb the healthy gut microbiome and its function, thus providing pathogens such as *Clostridioides difficile* a niche to colonize and cause disease. *C. difficile* is the leading cause of hospital-acquired infections today, with nearly half a million cases per year and approximately 29,000 deaths directly linked to *C. difficile* infection (CDI). Approximately 30% of patients that suffer from the disease can have multiple episodes of CDI recurrence and one treatment option, fecal microbial transplantation (FMT), is the most effective method of stopping the cycle of recurrent CDI. FMTs are thought to restore the homeostasis within the gut environment that resists *C. difficile*; however, human FMTs have yet to be regulated by the Food and Drug Administration (FDA) and may pose safety concerns to human health due to their complex composition of bacteria, archaea, viruses, helminths and fungi. While FMTs are an effective therapy for recurring CDI, there are initial indications that acute and chronic disease can be transferred to patients via FMT and the long-term effects on human health are unknown. The focus of this study was the development of a safe, defined, and simple microbial community that can be used to combat CDI. Using mini bioreactor arrays several human fecal samples were screened for *C. difficile* invasion resistance. Through further dilution and rescreening for invasion resistance, two simple fecal communities were identified. Illumina Miseq sequencing and analysis revealed that these microbial communities consist of 15-20 members. An in vivo model of CDI was conducted in Humanized microbiota mice (Hmbmouse) where mice were treated with the simplified communities prior to infection with a clinically relevant ribotype of *C. difficile* (ribotype 027). Results demonstrated that treatment protected against CDI by delaying symptoms and weight loss by one day and reduced *C. difficile* load measured in feces. Post recovery period of the initial infection, a relapse of CDI was induced by intraperitoneal injection of clindamycin. Mice that were treated with the simplified communities in the initial infection did not show a loss in body mass and had significantly lower *C. difficile* load in the relapse period of CDI. Overall results from in vitro and in vivo studies showed that simplified communities resisted *C. difficile* invasion and reduced CDI severity overall. In conclusion, the simplified microbial communities may serve as a therapeutic alternative to FMT treatment in CDI.

Conflict of interest: No

P 41

Incidence, clinical characteristics and outcome of *Clostridioides difficile* infections in the Netherlands in the period of May 2017-May 2018

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The National *Clostridioides* (*C.*) *difficile* Reference Laboratory was founded in the Netherlands in 2005, after multiple outbreaks with the hypervirulent *C. difficile* PCR ribotype (RT) 027. Since then, the reference laboratory has offered ad hoc typing service for all microbiology laboratories in the Netherlands for typing of *C. difficile* isolates of patients with severe disease, or isolates from a suspected outbreak. Additionally, the National Reference Laboratory initiated a sentinel surveillance programme in May 2009 to monitor the incidence of *C. difficile* infections (CDI) in 22 hospitals in the Netherlands. The results from the sentinel surveillance from the period May 2017 until May 2018 are presented here.
Clinical characteristics and 30-day outcomes of hospitalized patients (>2 years old) that are diagnosed with CDI in participating hospitals are registered in the Dutch sentinel surveillance. Severe CDI is defined as: hypoalbuminemia (<20 g/L) and/or dehydration, fever (temperature of 38°C or higher) and leucocytosis (>15×109/L), pseudomembranous colitis or bloody diarrhoea. A complicated course is defined as: admission to an intensive care unit, the need for surgical procedure or death. The participating hospitals send all *C. difficile* isolates to the Reference Laboratory for further characterisation.
in the period between May 2017 and May 2018, 879 patients with CDI were included in the sentinel surveillance. The mean incidence rate of CDI was 2.90 CDI cases per 10.000 patient-days, which was similar to previous years. The proportion of patients with severe CDI and the proportion of patients with a complicated course were also comparable to previous years. Severe CDI was seen in 20.4% of the patients. Of 797 patients with a reported 30-day outcome, 86.8% had an uncomplicated course, 0.4% needed surgery as a consequence of CDI, 0.3% was admitted to the ICU because of CDI and 12.5% of the patients died within 30 days (n=100). Twenty five deaths (3.1%) were due or contributable to CDI. Compared to the start of the surveillance, symptoms of CDI had more frequently

a community-onset. This was seen in 45% of the cases, while healthcare facility onset of symptoms was seen in 55% of the cases.
The most prevalent RT that was found was RT014/020 (20.9%), similar to the previous year. The proportion of CDI cases due to RT002 had increased significantly compared to previous years and was now the second most frequently encountered RT (11.9%). The hypervirulent RT027 was found in 1.2% of samples, which was similar to previous years.
Compared to previous years, the mean incidence of CDI has not changed in the Netherlands. The proportion of community-onset cases has increased compared to the start of the surveillance.
Severe CDI was seen in 20.4% of the patients and the overall mortality rate within 30 days was 12.5%. The CDI-related mortality rate was 3.1%. With a proportion of 20.9%, RT014/020 was the most frequently encountered RT in the Netherlands.

Conflict of interest: No

P 42

PCR ribotype 023: a new hypervirulent PCR ribotype of *Clostridioides difficile* in the Netherlands

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Clostridioides (C.) difficile PCR ribotype (RT) 023 appears to have similar molecular characteristics as the well-known hypervirulent strains RT027 and RT078, including a mutation in a toxin regulator gene and the presence of binary toxin genes. However, the severity and the outcome of *C. difficile* infections (CDI) with RT023 are not known.
Since 2009, 24 hospitals participate in the Dutch national sentinel surveillance program. Here, clinical characteristics of CDI due to RT023, compared to other ribotypes, are presented using data of the National Reference laboratory for *C. difficile* in the Netherlands.
Clinical characteristics and 30-day outcomes of hospitalized patients (>2 years old) that are diagnosed with CDI in participating hospitals are registered in the Dutch sentinel surveillance. Simultaneously, *C. difficile* isolates are sent to the National Reference laboratory.
All ribotyped isolates were divided into 6 groups: RT023, RT078/126, RT027, RT 014/020/295, RT001 and all ribotypes (except for RT023, RT027 and RT078/126). Clinical characteristics and outcomes were compared between RT023 and the other groups. Severe CDI were defined as: hypoalbuminemia (<20 g/L) and/or dehydration, fever (temperature of 38°C or higher) and leucocytosis (>15×109/L), pseudomembranous colitis or bloody diarrhoea. A complicated course was defined as admission to an intensive care unit, the need for surgical procedure or death.
In the period between May 2009 and February 2018, 5,359 patients with CDI were included in the sentinel surveillance. The proportion of RT023 was 2.4% (95% CI 2.0-2.8), encompassing 141 cases of CDI. CDI due to RT023 caused more severe disease, compared to CDI due to non-hypervirulent ribotypes, The severity of CDI was comparable to the hypervirulent RT027 and RT078/126 strains. Furthermore, CDI-related mortality of RT023 was similar to RT027 and RT078/126. Patients

with CDI due to RT023 had more frequently a community onset of symptoms. Four sequenced RT023 strains contained a large transposon region and an unique trehalose genotype.
CDI due to *C. difficile* RT023 leads to a clinical presentation with similar severity and results in comparable CDI-mortality as CDI due to the hypervirulent RT027 and RT078/126 strains. Furthermore, patients with CDI due to RT023 present more frequently with a community onset of symptoms.

Conflict of interest: No

P 43

Understanding the sequence diversity of the *Clostridium difficile* virulence factors TcdA and TcdB: impact on vaccine development

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Clostridium difficile is the most commonly recognized cause of infectious diarrhea in healthcare settings and represents a significant unmet medical need worldwide. Currently there is no vaccine to prevent initial or recurrent C difficile infection (CDI). Two large clostridial glucosylating toxins, TcdA and TcdB, are the primary virulence factors for CDI. Pfizer's investigational *C. difficile* vaccine, which consists of a mixture of genetically and chemically inactivated *C. difficile* toxoids A (TxdA) and B (TxdB), is currently in phase III clinical trials. An understanding of the sequence diversity of the two toxins expressed by disease causing isolates is critical for the interpretation of the immune response to the vaccine antigens. Traditional molecular typing approaches for *C. difficile* disease epidemiology studies (e.g., ribotype, MLST) do not provide insight to toxin variant diversity. In this study, we determined the whole genome sequence (WGS) of >500 C difficile isolates collected in 12 countries between 2004-2018 to probe toxin variant diversity. A total of 39 unique TcdA variants and 40 unique TcdB variants have been identified. Each of the TcdA variants shares at least 98% amino acid sequence identity with the TxdA antigen. Sequence diversity among the TcdB variants is more substantial, ranging from 86.1% to >99% identity with the TxdB antigen. Phylogenomic analysis of the WGS data demonstrate that isolates grouped together based on ribotype or MLST code for multiple different toxin variants. These findings illustrate the importance of determining the toxin sequence when evaluating immunological approaches to prevent *C. difficile* infections and that the current typing systems used are inadequate for this purpose.

Conflict of interest: Yes

Disclosures: Pfizer employee and may hold stock in the company

P 44

Isolation and Molecular Characterization of *Clostridium difficile* Strains from Patients with Diarrhea

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Clostridium difficile infection (CDI) is a leading cause of illness ranging from mild diarrhea to severe inflammation such as pseudomembranous colitis and ultimately leading to death. Currently, the prevalence and severity of CDI has increased rapidly due to the overuse and misuse of antibiotics and the emergence of hypervirulent strains. The aims of this study were to determine the molecular epidemiology and antimicrobial susceptibility of *C. difficile* isolates from hospitalized diarrheal patients admitted to the University-affiliated hospital. The presence of *C. difficile* in the stool samples was investigated by the enrichment multiplex-PCR procedure. Of 563 stool samples, 79 were shown to be PCR-positive. Twenty-six isolates of *C. difficile* were recovered from 79 PCR-positive stool samples using selective culture method. Four toxin types were found among 26 *C. difficile* isolates, which the *tcdA*+ *tcdB*+ *cdtA*/*cdtB*+ type was a predominant group. Sixteen different PCR-ribotype patterns were classified. The ribotype 017 was the most common type, accounting for 26.9% of *C. difficile* strains isolated in the hospital. The antibiotic susceptibility profile of *C. difficile* isolates was studied. All *C. difficile* isolates showed highest resistance to chloramphenicol (100%). Resistance frequencies to cefoxitin, levofloxacin, rifampicin, vancomycin and metronidazole were 92.3%, 76.9%, 30.7% 11.5%, and 3.8%, respectively. Altogether, 21 (80.8%) isolates were characterized as multidrug resistant (MDR) as they acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Conflict of interest: No

P 45

Identification of a host receptor for *Clostridium difficile* specific bacteriophage, ΦHN10.

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Recognition and absorption of phages onto the host are crucial for the successful infection of bacteriophages. Thus, insights on the mechanism of absorption and recognition are extremely important to the investigation of host-phage interaction, host range determination and the coevolution processes of both partners. Here, we identified and characterized a potential phage host receptor involved in the absorption of *Clostridium difficile* ΦHN10. Interestingly, no obvious alteration in the adherence of ΦHN10 phage was observed in the pili and flagellum disrupted cells. Further electron microscopic analysis of the *C. difficile* interacting with the ΦHN10 phages clearly revealed strong attachment between phage and array of *C. difficile* cell wall protein, potentially the S-layer protein, of both intact and appendages-free cells. The electrophoretic mobility shift assay revealed a clear interaction between isolated phage, ΦHN10 and the S-layer proteins, confirming the role of *C. difficile* S-layer protein as a phage receptor of ΦHN10. Our results thus provided the foundation for further investigation of complex interaction between *C. difficile* phages and their hosts.

Conflict of interest: No

P 46

Elucidating the regulatory network of chaperone expresson during bile acid challenge in *Clostridioides difficile*

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Clostridioides difficile is an increasingly important nosocomial pathogen, which colonizes the gut mainly after antibiotic treatment causing diarrhea and in severe cases pseudomembranous colitis. An important aspect of the infection is the adaptation of *C. difficile* to the conditions in the gastrointestinal tract, e.g. a high concentration of bile acids. *In vitro* assays elicited a general cellular stress response after 90 minutes exposure to different bile acids (Sievers et al., 2019). The amphiphilic nature of bile acids also causes denaturation of proteins resulting in an increased abundance of chaperones: the protein amount of DnaK, DnaJ, and GrpE (encoded in one operon) increases in response to all tested bile acids (cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA) and lithocholic acid (LCA)), while GroL and GroS, ClpB, the arginine kinase McsB and the ATPase ClpC were increased only upon LCA-treatment and to a lesser extend upon CDCA treatment. Although chaperones have been in the focus in heat shock analyses in *C. difficile* (Ternan et al., 2012), the exact regulatory mechanisms of different chaperones of *C. difficile* has not been described yet.
To elucidate the mechanistic details on the specific expression of chaperones upon challenge with different bile acids to eventually obtain a comprehensive picture on chaperone regulation of *C. difficile*.
C. difficile 630 cultures were grown to A600 0.4 prior to a challenge with four different bile acids allowing continuous growth with half maximal rate (4.5 mM CA, 0.4 mM DCA, 0.4 mM CDCA and 0.08 mM LCA). A control without bile acid was included. RNA was isolated from cells harvested after 30 minutes and 60 minutes bile acid stress. RNA was reversely transcribed into cDNA and selected chaperones quantified by qRCR.
A treatment with different bile acids revealed the differential expression of chaperones as shown in previous proteomic studies (Sievers et al., 2019). In this work, we analyzed whether the different regulation on protein level is based on transcriptional differences of chaperone genes pointing at an involvement of several transcriptional regulators.

Conflict of interest: No

P 47

The phi027 prophage increases toxin production in the *Clostridioides difficile* strain R20291 and other ribotype 027 epidemic isolates

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Most *Clostridioides difficile* (*C. difficile*) strains are susceptible to bacteriophage (phage) infection. Our recent bioinformatic analyses on thousands of *C. difficile* genomes allowed us to identify prophages (phages integrated into their host chromosome) in virtually every strain analyzed. This observation underlines the extent of the phage-host relationship in *C. difficile* and calls for a better understanding of the potential role played by phages in the biology and virulence of this important pathogen.

The ribotype 027 lineage (R027) represents a very important group of clinical isolates responsible for major outbreaks worldwide. R027 isolates generally produce more toxins than other isolates and are often described as being "hypervirulent". Preliminary bioinformatic analyses of *C. difficile* genomes suggested that a particular prophage, phi027, was always present in R027 isolates, but rarely found in other ribotypes. Hence, phi027 seemed to be highly conserved and specific to that lineage. It was therefore tempting to hypothesize that phi027 contributes to the virulence of R027 isolates.

To study this question, we first inspected 2,800 R027 genomes and found that very few (N=6) lacked the phi027 prophage, thus confirming its highly conserved nature. Mitomycin C was then used to induce the phi027 prophage from its original host, the R20291 strain, to produce infectious particles. Next, the phage was reintroduced into 4 of the six sensitive R027 isolates lacking phi027 to create new lysogens.

Intra and extracellular culture fractions were collected after 4 and 8 hours of growth, and toxin production was assessed by ELISA and toxicity assays on VERO cells. Preliminary results revealed that new lysogens carrying phi027 secreted up to 6-fold more toxin A compared to cells lacking the prophage (ELISA at 8 hours). A 16-fold increase in toxin activity was also observed using VERO cell culture assays. Together, our data suggest that phi027 increases toxin production when introduced into other R027 strains.

In parallel, we deleted the entire phi027 prophage from the genome of the wild-type R20291 strain using an allelic exchange approach. Toxin production was assessed and as expected, we observed a 4-fold lower toxin secretion in the cured strain lacking phi027, and the phenotype was restored upon re-lysogenization with phi027.

Altogether, our preliminary results show that phi027 increases toxin production in the R20291 strain and possibly in all other R027 isolates in which it is integrated, thus contributing to the virulence of this important lineage. Additional experiments are underway to confirm our results, including qRT-PCR determination of PaLoc gene expression. Infection assays in mice will be performed to assess whether the increased toxin production observed *in vitro* also occurs *in vivo*, and whether it translates into more complicated symptoms and issues.

Conflict of interest: No

P 48

Signature sequences of Clade C-I *Clostridium difficile* strains

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Clostridium difficile is a highly diverse species. Whereas the MLST clades 1 through 5 include strains of common association with human infection, the so-called cryptic clades C-I, C-II, and C-III contain highly divergent strains, most of which are non-toxigenic. There have been sporadic reports of human infection by toxigenic strains from the Clade C-I and in Costa Rica their isolation rate is on the rise. However, these bacteria are unreliably detected by widespread diagnostic methods and therefore can remain undiagnosed. Aiming to i) identify potential targets for robust detection of these emerging strains and ii) better understand their divergence at the genomic level, we used an average nucleotide identity test (FastANI), pangenomics (anvio), and a genome-wide association analysis (scoary/roary) to compare 10 whole genome sequences (WGS) of Clade C-I strains (inclusion group; IG) and 67 WGS of strains representing clades 1 to 5 (exclusion group; EG). While IG vs EG ANI values ranged from 90.6 to 91.4, cognate values obtained for EG strains from clades 1 to 5 were always above 95. Around 3% of the pangenome CDS delimited by roary (600/18952) were associated with the IG trait with 100% sensitivity and specificity (Bonferroni corrected p=1,59892e-08). When classified in gene clusters, nearly half of the anvio IG hits (45/89) corresponded to genes for metabolic enzymes or, to a lesser extent, transport proteins and transcriptional regulators. We are currently evaluating the specificity of markers identified by both approaches to continue with the design of a comprehensive qPCR-based detection technique. Our results urge for a revised classification of the Clade C-I strains and highlight marked differences in their metabolic potential.

Conflict of interest: No

P 49/FL 1

Membrane vesicle-mediated immunogenic protein secretion in *Clostridium perfringens*

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Membrane vesicles (MVs) are 20-400 nm spherical structure derived from cell envelope membrane. Many bacteria release MVs that carry a wide range of cargos including nucleic acids, proteins, lipids and quorum-sensing signals. Therefore, MV plays an important role in the bacterial or bacterium-host cell-cell interactions. Previously we have shown that *Clostridium perfringens* actively releases MVs that induce the inflammatory cytokine secretion of host cells via Toll-like receptor 2 (TLR2) signaling pathway (Obana et al. 2017. Infect. Immun.). However, which component(s) of MV is involved in the induction of host immunity is still unclear. In present study, we found that nasal vaccination of purified MVs to mice induces the production of *C. perfringens* cell-specific serum IgG and secretory IgA antibodies. These results indicate that MVs also induce the host acquired immunity as well as innate immunity. We identified two hypothetical lipoproteins LipO and MalE as the major proteins contained in *C. perfringens* MVs by SDS-PAGE and MALDI TOF-MS. We confirmed that these fluorescent fusion proteins, LipO-RFP and MalE-RFP were predominantly localized at cell surface and MVs by confocal laser scanning microscopy. In addition, administration of MVs to mouse nasal cavities elicited high level of serum IgG and fecal IgA antibodies against LipO. Since lipoprotein is one of the ligands of TLR2, LipO enriched in MVs is highly likely to be involved in MV immunogenicity. These results suggest that *C. perfringens* MVs deliver LipO protein, which acts as an immunodominant antigen to induce host immunity. MVs would be closely involved in pathogenicity and *C. perfringens* host colonization, and may be applied for the development of vaccine platform.

Conflict of interest: No

P 50

Microbiome recovery following antibiotic exposure – trehalose supplementation is not associated with simulated CDI induction

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Within the human intestinal tract, dietary, microbial- and host-derived compounds are used as signals by many pathogenic organisms, including *C. difficile*. The presence (and potentially absence) of these environmental cues are critical for induction of gene expression to bring about a change in the cell lifestyle. For example, primary and secondary bile acids have different effects on the germination of *C. difficile* spores, whereas microbial derived succinate is utilized *C. difficile* vegetative cells to gain a growth advantage. The role of dietary signals, specifically trehalose, are less well defined; it has been reported the presence of trehalose confers a growth/virulence advantage to those *C. difficile* strains that harbor the ability to metabolise trehalose.
We used three clinically reflective human gut models to simulate the effects of either trehalose, glucose or saline supplementation on the microflora (including *C. difficile* spores). Pooled human faecal samples were seeded into 3 parallel gut models, which were supplemented with either trehalose, glucose or saline and inoculated with *C. difficile* RT027 spores. Simulated CDI was induced with a clindamycin regimen and the recovery of the microflora and CDI induction monitored in each of these models. Here we measured the bacterial changes (by direct plating and 16S taxonomic analysis), *C. difficile* cytotoxin analysis and in vitro trehalose/glucose concentration.
A stable and diverse microflora prevented *C. difficile* spore germination, which was unaffected by either sugar or saline supplementation. During clindamycin instillation, reductions in many bacterial populations, such as *Clostridium* spp., *Bifidobacterium* spp. and *Bacteroides* spp. were observed, which coincided with an accumulation of trehalose in vessel 1. Simulated CDI was detected in the saline or glucose supplemented models 9-10 days after withdrawal of clindamycin, whereas, despite germination and outgrowth of spores during this same time, no toxin was detected in the model supplemented with trehalose. The lack of

simulated CDI in our model was associated with earlier recovery of microflora compared with the saline supplemented model. Higher levels of glucose were detected in the trehalose model compared with saline model, suggesting microbial breakdown of trehalose did occur.
Microbial and host metabolism of trehalose could prevent trehalose accumulation in the colon where CDI most likely occurs. Our data show that trehalose supplementation was associated with a lack of *C. difficile* toxin detection (simulated CDI) in our model, which correlated with enhanced recovery of the microflora and a decreased accumulation of trehalose post antibiotic exposure.

Conflict of interest: Yes
Disclosures: This is a collaborative study with HAYASHIBARA CO., LTD., whom provided the trehalose and glucose chemicals used in this study and evaluated trehalose and glucose concentrations from the gut model samples. They played no role in study design or the experimental outcomes.

P 51

Chlorotonil A and B as new therapeutic options for *Clostridioides difficile* therapy

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Disruption of the intestinal microbiota by antibiotics can promote an infection with the nosocomial pathogen *Clostridioides difficile*. Consequently, antibiotics that spare the intestinal microbiota and antibiotics that can selectively kill *C. difficile* without further harming the intestinal microbiota are urgently needed.
Our multi-omics approach aims at characterizing the macrocyclic compounds Chlorotonil A and B with regard to their mode-of-action and their global effects on the intestinal microbiota to evaluate the two substances as possible therapeutic options for *C. difficile* infections.
First, human and porcine *C. difficile* isolates were tested for their susceptibility to Chlorotonil A and B. Subsequently, *C. difficile* was exposed to sublethal concentrations of Chlorotonil A and protein samples of stressed and unstressed cells were analyzed by gel-free LC-IMSE. In addition, Chlorotonil A was administered to piglets. Feces of the piglets were analyzed by 16S rRNA gene sequencing and are currently further processed in an integrated metatranscriptomics-/proteomics approach.
Chlorotonil A and B and their derivatives show significant activity against *C. difficile* isolates (MIC range: 0.1 to 6.4 µg/ml). Data from the LC-IMSE analysis are currently analyzed and will give an insight into the cellular stress response of *C. difficile* towards Chlorotonil A and will thereby potentially provide valuable knowledge on the mode-of-action of the substance. Besides, preliminary results from the animal experiment already indicate that Chlorotonil A might be less harmful to the intestinal microbiota than our reference antibiotic Tylosin (macrolide antibiotic). Susceptibility testings on key members of the intestinal microbiota, including *Clostridium scindens* and *Terrisporobacter glycolicus*, and results of the meta-omics analysis will hopefully confirm this hypothesis.
Given the potentially unique mode-of-action of Chlorotonil A and B and their hitherto observed safety with respect to intestinal dysbiosis, Chlorotonil A and B

might be promising candidates for patients at high risk for *C. difficile* infections as well as for those already infected by *C. difficile*.

Conflict of interest: No

P 52

Shedding light on the interactomics underlying the Stickland fermentation of *Clostridioides difficile*

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The anaerobic, Gram-positive bacterium *Clostridioides difficile* is responsible for severe diarrhoea, causing significant morbidity and mortality worldwide among individuals of all ages [1]. Stickland fermentation of amino acids stands for the most prominent energetic generation process for *C. difficile* propagation. Importantly, this pathway has been unequivocally correlated to toxin production and sporulation [2]. This metabolic route employs pairs of amino acids as electron acceptor-donor systems for ATP generation. The proton motive force and its concurrent ATP synthesis originates at the proton-translocating ferredoxin:NAD+ oxidoreductase complex (Rnf), whose proton pump functioning is coupled to the course of action of amino reductases [3]. Our group is endeavoured towards the exploration of protein-protein-interactions occurring during the Stickland fermentation. On the pursuit of that purpose, interactomic techniques encompassing affinity co-purification of bait-prey protein complexes and proteomics-based analysis for the identification of interaction partners were selected as the scientific tactic. Firstly, the recombinant tagged variants of the cytoplasmic proline reductase (subunits PrdA and PrdB) as well as a tagged RnfC subunit of the membrane-residing Rnf complex were subjected to interactomic examination. Transient interacting preys were captured by in vivo cross-linking stabilization prior to cell lysis. Proper purification of the baits was verified by Strep-tagII immunodetection. LC-MS/MS analysis led to the identification of potential interaction partners mostly involved in electron transport and subsequent energy generation. Taking together the current interactions assessments we have glimpsed into the probable existence of a high-ordered membrane complex encompassing the Proline reductase and the Rnf pump.

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Conflict of interest: No

P 53

Bile acids and their differential action on gene expression and morphology in *Clostridioides difficile*

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Bile acids have been frequently reported to substantially influence the outcome of an infection with the intestinal and spore-forming pathogen *C. difficile*. The bacterium attains more and more attention due to an increasing number of patients and a dramatically high relapse rate. Different bile acids were reported to have specific effects on *C. difficile*, for instance taurocholic acid is an instigator of spore germination whereas other bile acids inhibit growth [1]. Specifically, intestinal commensal bacteria are able to convert primary into secondary bile acids, which can prevent a *C. difficile* infection [2]. However, details on the bile acid stress response of vegetative *C. difficile* cells on a molecular level are largely unknown.
To elucidate the molecular details on the action of different bile acids on *C. difficile* by tracking gene expression and changes in morphology and cellular membrane structure.
Changes in relative protein abundance were determined by proteomic analyses in *C. difficile* 630Δ*erm* that was long-term stressed with the four main bile acids cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and lithocholic acid (LCA). LC-MS/MS generated data were evaluated by using the MaxQuant/Perseus software packages. Morphological changes were analyzed in electron micrographs and by fluorescence confocal microscopy. Thin layer chromatography (TLC) was employed to determine changes in the lipid composition of the cellular membrane, and the localization of bile acids within *C. difficile* cells was traced by using bile acid specific antibodies.
Long-term challenge with four different bile acids rather induces specific stress responses than a general bile acid stress response. The major structural flagellum protein FliC was dramatically decreased in the presence of LCA compared to control conditions, while DCA and CDCA resulted in a moderate decrease in abundance of FliC, and CA did not affect FliC expression at all. These findings were not only confirmed by transcriptomic analyses, but could also be validated by microscopic analyses: while CA treatment did not alter the number of flagella, DCA- and CDCA-stressed *C. difficile* had fewer and LCA-treated bacteria were

almost devoid of flagella compared to controls. Differential *fliC* expression was reflected in motility and in adherence of *C. difficile* to Caco-2 cells. The detected morphological differences could result from specific structural adaptations in the cellular membrane as determined by TLC and from distinct cellular localization patterns of the single bile acids.

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Conflict of interest: No

P 54

Clostridium difficile LMW SLP contributes to acute disease in the mouse

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The intestinal immune system is constantly exposed to many different organisms, from commensals to pathogens, and presents an array of strategies to maintain homeostasis. For pathogens to thrive in such a hostile environment, protection against innate immune effectors is crucial, and it can be provided by surface components that maintain cell wall integrity. Accordingly, mechanisms of protection and evasion have been described for outer membranes, wall teichoic acids and capsules. However, the role of S-layer in pathogenesis remains to be clarified.
Clostridium difficile gut colonisation is associated with a wide spectrum of gastrointestinal diseases, ranging from mild to severe, acute to persistent. Pathogenicity and fulminant disease are mainly attributed to the production of Toxins A and B.
The S-layer is ubiquitous in *C. difficile* strains and, upon cleavage of the precursor protein SlpA, is composed of a conserved (HMW SLP) and a variable (LMW SLP) region. The LMW SLP confers strain specificity, is highly immunogenic and faces the external environment. The S-layer has been reported as an adhesion factor to the gut epithelia. In addition, we have shown that strain FM2.5, which does not produce S-layer, is avirulent in the Golden Syrian hamster model of *C. difficile* infection [1]. More recently, in vivo and in vitro evaluation of this strain has suggested that delayed toxin expression could have contributed to the absence of lethal disease. Our recent studies have focused on a derivative strain, RΔD2, that whilst containing a deletion in the LMW SLP, still presents an assembled S-layer at the cell surface. Mice infected with RΔD2 showed limited weight loss and disease in comparison to mice infected with the wild type strain, whilst still displaying high levels of colonisation and gut toxemia. Our results suggest that the S-layer contributes to pathogenicity by an as yet unknown mechanism. We hypothesise that the LMW SLP may trigger strong inflammatory responses, which contribute

to the severity of the disease.

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Conflict of interest: No

P 55

Necrotic enteritis pathogenesis: studying *Clostridium perfringens* using comparative genomics

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Necrotic enteritis (NE) in chickens and its causative agent, *Clostridium perfringens*, have captivated the mind of the scientific community over the past decade due to a worldwide resurgence of the disease resulting from both the ban and the reduction on the prophylactic use of antibiotics. NE occurrence is multifactorial and some of the pathogen virulence attributes appear to be the major contributing factors (1). Few studies have looked at the comparative genomic epidemiology of avian *C. perfringens* (2). In this study, 48 *C. perfringens* strains recovered from healthy and NE-affected broiler chicken flocks were whole genome sequenced and in silico analysed in order to explore and to compare their content in plasmid, phage, antimicrobial resistance and protein-encoding genes. *C. perfringens* genomes were sequenced using the Illumina platform. Raw sequences were submitted to the INNUca pipeline, followed by an annotation with IRIS from RASTtk. Genome assembly statistics were generated with QUAST. Bioinformatic tools such as ROARY, PlasmidSpades, PHASTER, and CARD were used for the identification of pan-genome plasmid, prophage region and antimicrobial resistance genes, respectively. CELLO and VaxiJen softwares performed the evaluation of protein localisation and immunogenic potential. The average total genomic content was 10 585 genes. Results revealed a highly heterogeneous genomic composition among all samples, with a whole genome length varying from 2.92 to 6.42 Mb, and a number of coding regions being as low as 2455 and as high as 5464. Whereas the core genome was made up of 163 genes that were commonly shared, 10 422 genes formed the 48-strain accessory genome and 1 490 genes were identified as unique. An average of 9 plasmid components were identified among the analysed genomes, with 75% of the strains possessing less than 10 components. A total of 24 phage or prophage regions were identified. While 8 of these regions were unique to specific strains, CP 51, phiSM101 and phi3626 regions were found in 67%, 47% and 28% of the genome sequences, respectively. The CELLO software identified a total of 155 700 protein-encoding sequences classified as 13% extracellular, 65% cytoplasmatic and 22% membrane-bound proteins. From the 89 protein sequences that were uniquely found in NE-associated strains, the VaxiJen prediction software identified 8 extracellular, 9 cytoplasmic, 10 membrane-bound and 5 unknown proteins with an immunogenicity score higher than 0.5. Investigating the contribution of these proteins in the virulence of NE-causing *C. perfringens* will help elucidate the disease pathogenesis.

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Conflict of interest: No

P 56

Comparison of virulence phenotypes of NAP1/027/ST1 and non-NAP1/027/ST1 Clade 2 *Clostridium difficile* strains

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The incidence and mortality of *Clostridium difficile* infections (CDI) have increased in the past two decades, in part due to the emergence and dissemination of highly virulent, epidemic types such as the NAP1/027/ST1 strain. This strain is classified by multilocus sequence typing (MLST) in the so-called Clade 2, along with other 65 STs that have not been implicated in outbreaks despite their close phylogenetic relationship. This observation suggests that virulence is a variable feature among Clade 2 members, with the NAP1/027/ST1 strain displaying a distinct phenotypic profile. We have isolated representatives of 6 STs from the MLST Clade 2 (ST01, ST41, ST47, ST67, ST154, and ST252) as a result of nearly 10 years of surveillance of *C. difficile* in hospitals from Costa Rica. Here we use this valuable resource, along with animal biomodels and a series of *in vitro* tests of relevance to various stages of the infection process, to experimentally clarify whether the NAP1/027/ST1 strain is indeed more virulent than non-NAP1/027/ST1 Clade 2 strains and thereby pinpoint key elements of its pathogenicity mechanism. In line with the working hypothesis, ST01, followed closely by ST67, displayed the highest scores of inflammation, tissue damage, and lethality in a murine intestinal loop model and a hamster infection model. By contrast, no clear trends were observed in i) the motility and biofilm-forming capacity of the isolates, ii) their amounts of surface-bound flagellin, secreted SlpA, and secreted TcdB, and iii) the potency, type of cytopathic effect elicited, and pool of small monomeric GTPases targeted by their culture supernatants. These unexpected results open avenues for further research. We are analyzing phenotypes related to sporulation and germination and will complement this characterization with a pangenomic comparison of whole genome sequences in search of traits with a potential diagnostic and therapeutic application.

Conflict of interest: No

P 57

Electrochemically activated dissolutions for disinfection of surfaces contaminated with *Clostridium difficile* spores

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The ability of *C. difficile* endospores to withstand commonly used disinfectants sets hurdles to their control in the hospital environment. Oxidizing and chlorine-releasing agents such as hypochlorite (ClO-1) and sodium dichloroisocyanurate (NaDCC) have proven effective to kill *C. difficile* spores. Nonetheless, their use is associated with numerous drawbacks, including surface damage through corrosion, insecure handling, bad odor, and irritation to eye, skin, and mucous membranes. Based on their potent bactericidal and sporicidal activity, low production costs, point-of-use preparation, and environmental compatibility, electrochemically activated solutions (ECAS) emerge as attractive alternatives. In this study, we compared the efficacy of a NaCl-derived ECAS (0.18% w/v ClO−, pH=9.6-10.3), household bleach (2.83% w/v ClO−, pH=5.6), and NaDCC (0% w/v ClO−, pH=6.8) to inactive *C. difficile* spores on contaminated inanimate surfaces using a standardized quantitative disk carrier test (US EPA MO-21-03) and local strains from MLST Clades 1 to 5 recovered from humans. The logarithmic reduction values (LRV) obtained revealed significant differences between the treatments (F2,15=15.41, p=0.0002), the strains tested (F4,15=27.21, p<0.0001), and the interaction between both factors (F8,15=3.752, p=0.013). ECAS and household bleach showed comparable performances, with LRVs ranging from 0.72 to 4.08 and 0.71 to 4.50, respectively. NaDCC, by contrast, was associated with LRVs between 0.46 and 2.17. Though some strains were resistant to the treatments, our results indicate that NaCl-ECAS hold some promise for disinfection of *C. difficile* on hard, nonporous environmental surfaces and medical devices.

Conflict of interest: No

P 58

Functional and structural analysis of the two families of conjugative toxin and antibiotic resistance plasmids from *Clostridium perfringens*

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For many years it was considered that plasmids related to the tetracycline resistance plasmid pCW3 and the CPE plasmid pCPF4969 represented the only conjugative plasmid family in *Clostridium perfringens*. Members of this plasmid family carry the *tcp* conjugation genes and it is proposed that they be referred to as Tcp plasmids. We have now shown that pCP13, which carries a cpb2 toxin gene, is also conjugative. Genetic studies have provided evidence that the conjugative transfer of pCP13 is mediated by the pcp conjugation genes, which are not closely related to their tcp homologues. It is proposed that members of this conjugative plasmid family be referred to as the Pcp plasmids. The Pcp plasmid family includes pCP-TS1 and pCP-OS1, both of which encode the BEC binary enterotoxin. pCW3 has been used as the paradigm Tcp plasmid for genetic and structural studies on the conjugation and plasmid maintenance processes. Extensive mutagenesis studies have led to the identification of at least nine genes that are required for efficient conjugative transfer. Two key components of the conjugation apparatus have been identified: the coupling protein, TcpA, and the unique relaxase protein, TcpM. The origin of transfer (oriT) site has been characterized and the structure of the relaxase accessory protein, TcpK, has been determined in the presence and absence of oriT DNA. We have recently determined the structure of the soluble component of the hexameric ATPase TcpA in the presence and absence of ATP. The results show that TcpA is more closely related to FtsK, which is involved in DNA segregation at cell division, than to coupling proteins from other conjugation systems. We have also used super-resolution microscopy to image components of the Tcp apparatus and have shown that the conjugation machinery is located in the cell envelope, whereas relaxase components are cytoplasmic. Finally, both genetic analysis and biophysical studies using surface plasmon resonance have shown that the ability of a single *C. perfringens* cell to carry up to five different Tcp toxin plasmids is dependent upon the specific ParMRC partitioning system carried by each of these plasmids. In conclusion, these studies have significantly

advanced our understanding of how toxin and antibiotic resistance plasmids are maintained and spread in *C. perfringens*.

Conflict of interest: No

P 59

The improvement of gut health and protective effect against *Clostridium difficile* by probiotic bacteria isolated from Thai fermented sausages.

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Clostridium difficile is one of the major causes of nosocomial diarrhea ranging from mild diarrhea to life-threatening pseudomembranous colitis. Due to its multidrug resistance, alternative methodologies including the use of probiotic bacteria such as lactic acid bacteria have been studied for prevention and protection of *C. difficile* infection. Two lactic acid bacteria, *Enterococcus faecium* and *Lactococcus lactis*, isolated from Thai fermented sausages were investigated their probiotic properties especially in the protective effect against *C. difficile* 630. The results showed that both isolates could survived in simulated gastric solution at pH3 followed in simulated intestinal solution at pH8. They also could adhere to Caco-2 cell and their neutralized cell free supernatant exhibited no cytotoxicity to the cell. Since *C. difficile* produces toxin A and toxin B that play important roles in the disruption of the actin cytoskeleton resulting the damage of the intestine, cell-free supernatant of *C. difficile* 630 exhibited high toxicity to the Caco-2 cell. Moreover, it induced high expression level of pro-inflammatory cytokines which are tumor necrosis factor-alpha and interleukin-8 investigated by quantitative reverse transcription PCR. However, the presence of neutralized cell free supernatant of both *E. faecium* and *L. lactis* isolates could reduce the expression level of TNF-alpha and IL-8 induced by the cell-free supernatant of *C. difficile* 630. Hence, both isolates would be developed to be used as probiotic bacteria and would be able to improve the gut health through counteracting the *C. difficile*-associated intestinal inflammation in human cell lines .

Conflict of interest: No

P 60

Autolysin and GAPDH as the functional fibronectin-binding proteins on the *Clostridium perfringens* cell surface

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Fibronectin (Fn) is one of the extracellular matrix proteins, found in plasma and in connective tissues, playing a pivotal role in wound healing and cell adhesion. The involvement of Fn in the microbial infection has been suggested by the evidence that many microorganisms have the Fn-binding proteins (Fbps) on their cell surfaces. *Clostridium perfringens*, the cause of gas gangrene and food poisoning, is known to have the peptidoglycan-associated Fbps including FbpC (CPE0625), FbpD (CPE0630), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, CPE1304). GAPDH, a glycolytic enzyme, was reported as being expressed on the *C. perfringens* cell surface probably by interaction with the autolysin (Acp, CPE1231) that is a cell wall associated enzyme [1].

During the investigation of the Fn-binding activity of GAPDH associated with Acp, we found that Acp itself bound Fn, indicating that Acp is also an Fbp. The Fn-binding activity of Acp was, however, strongly inhibited by the binding of GAPDH to Acp. We tried to identify the functional Fbp(s) among these four Fbps: FbpC, FbpD, GAPDH, and Acp. Because the mutant *C. perfringens* cells (SAK3), lacking both FbpC and FbpD, had a similar Fn-binding activity as the wild type cells (HN13), we focused on the Acp and GAPDH. The Fn binding to SAK3 cells was inhibited by the presence of FbpD, GAPDH, and Acp, but not by FbpC. FbpD was, however, found to bind to both GAPDH and Acp. Treatment SAK3 cells with either anti-GAPDH or anti-Acp antibody suppressed the Fn binding to SAK3 cells. Thus, both GAPDH and Acp, probably the complex of them, are the functional Fn-binding proteins for the soluble Fn. On the other hand, Fn is known to bind to collagen. SAK3 cells as well as HN13 cells bound to the Fn-prebound collagen (collagen-Fn), but not or little to collagen [2]. SAK3 cell binding to collagen-Fn was not only inhibited by the presence of either GAPDH or Acp but also by anti-Acp antibody, but not by anti-GAPDH antibody. Moreover, Acp bound to collagen-Fn whereas GAPDH did not. Taken together, although the GAPDH-Acp complex on the surface of *C. perfringens* cells is involved in the interaction with collagen-Fn, the Acp parts of GAPDH-Acp complex may interact directly with collagen-Fn.

[1] N. Matsunaga et al. 2018 Anaerobe 51:124-130.
[2] Y. Hitsumoto et al. 2014 Anaerobe 25:67-71.

Conflict of interest: No

P 61

Fibronectin-binding proteins (FbpA and FbpB) from *Clostridium perfringens* inhibit the binding of fibronectin to dermatopontin

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Fibrillation of fibronectin (Fn) is important in the wound healing process. To date, III1-C, a fragment of Fn derived from the carboxyl-terminal two-thirds of the first type III module, and dermatopontin (DPT) are known as Fn-fibrillation promoting factors.

Dermatopontin (DPT) is a 22 kDa non-collagenous extracellular matrix protein, having multiple activities including the induction of Fn-fibrillation that is necessary for wound healing. DPT induces Fn-fibrillation via the binding of the type III12-14 region of Fn to DPT.

We identified two Fn-binding proteins (Fbps) of *Clostridium perfringens* cells, named as FbpA (CPE0737) and FbpB (CPE1847). Both FbpA and FbpB inhibited the binding of Fn to III1-C by binding of them to III1-C. III1-C is, however, known to be a cryptic site in serum Fn. The biological significance of the inhibitory effects of both FbpA and FbpB on the III1-C-induced Fn-fibrillation is unclear. DPT is known to bind III1 as well as III12-14. Therefore, FbpA and/or FbpB were expected to affect DPT-induced Fn-fibrillation.

We investigated the effects of FbpA and FbpB on the binding of Fn and III12-14 to DPT by using enzyme linked immunosorbent assay. Both recombinant FbpA (rFbpA) and recombinant FbpB (rFbpB) significantly inhibited not only the binding of Fn to DPT but also the binding of recombinant III12-14 (rIII12-14) to DPT. rFbpA and rFbpB interacted with DPT but not with rIII12-14. These results suggested that the binding of Fn to DPT was inhibited by the presence of FbpA and FbpB.

FbpA and FbpB may involve in a delay of wound healing by the inhibition of DPT-induced Fn-fibrillation.

Conflict of interest: No

P 63/FL 3

Quorum sensing controls heterogeneity and biofilm morphological change in *Clostridium perfringens*

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Clostridium perfringens is known as a causative agent of gas gangrene and food poisoning. The toxin production of this organism is regulated by agr-like (accessory gene regulator) quorum sensing (QS). QS contributes to environmental adaptation in response to cell density for host infection. In addition, *C. perfringens* can adapt to the environment by biofilm formation that increases tolerance to environmental stresses such as desiccation and oxygen. Biofilm consists in cells and self-produced extracellular polymeric substance (EPS) that provide the mechanical stability of the biofilm. In the previous study, we reported that *C. perfringens* forms biofilms with a different morphology at different temperature (Obana et al. (2014) J. Bacteriol.). At 25°C cells produce elastic pellicle-like biofilm as the threadlike extracellular matrix production is increased at 25°C, compared to 37°C, where cells densely attach to the surface. The morphological change of biofilms is predicted as an adaptive survival strategy between the inside and outside of the mammalian host, and thus both QS and biofilm formation plays an important role in pathogenesis of this organism. However, the involvement of QS on biofilm formation is still poorly understood in *Clostridium*. Therefore, in present study, we decided to analyze the relationship between QS and biofilm formation at the single-cell level, using the fluorescence promoter-reporter strains.
We constructed fluorescent reporter strain of threadlike extracellular matrix-producing gene cluster (*sipW* operon) that is necessary for pellicle biofilm formation at 25°C. FACS and fluorescent microscopy analysis of the reporter strains show that the sipW promoter displays bimodal expression in wild type strain population. In contrast, in the mutant strain of *agrBD genes,* which is responsible for QS signal peptide synthesis, the *sipW* promoter expression is activated in the whole population. Moreover, *ΔagrBD* loses the ability for the morphological change of biofilms in response to temperature as the cells produce pellicle-like

biofilm at any temperature. Co-culture of ΔagrBD with wild type strain restores bimodal expression of *sipW* promoter in *ΔagrBD.* These results suggest that intercellular delivery of QS signals regulates the morphological change of biofilms by suppressing the expression of *sipW* operon. In addition, although VirRS-VR-RNA cascade is known to contribute to the regulation of toxin gene expression by agr-like QS in *C. perfringens*, *ΔvirRS* cells still display the bimodal expression of *sipW.* Our results imply that suppression of EPS production by *agr*-like QS is mediated by a novel signaling pathway other than VirRS-VR-RNA cascade. We suggest that *Clostridium* QS plays a important role for their pathogenesis through regulation of biofilm formation as well as toxin production.

Conflict of interest: No

P 64

Negative control of sporulation initiation in *Clostridium difficile*

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Bacteria that reside in the gastrointestinal tract of healthy humans are essential for our health, sustenance and well-being. About 50 to 60% of those bacteria have the ability to produce resilient spores, important for the life cycle in the gut and for host-to-host transmission. Recently a genomic signature of sporulation within the human intestinal microbiome was described. We report on the identification of a new gene, termed *csiA*, part of the genomic signature for sporulation in the gut, which is involved in the control of sporulation initiation in *Clostridium difficile*. We show that CsiA accumulates during growth. An in-frame deletion of csiA results in increased sporulation. The key regulatory protein for entry into sporulation is *Spo0A*, which is activated by phosphorylation. We show that deletion of *csiA* increases the expression of *spo0A* per cell, and also the expression of the Spo0A-dependent *spoIIG* operon, coding for the first mother cell specific RNA polymerase sigma factor, σE. In contrast, deletion of *csiA* did not increase the expression of the Spo0A-dependent spoIIA operon, coding for the first forespore-specific sigma factor, σF. Increased expression of *spoIIG* results in increased production and proteolytic activation of pro-σE, suggesting that the levels of active σE are limiting for sporulation. Deletion of the *csiA* gene does not alter the expression of the genes coding for the TcdA and TcdB cytotoxins, or the genes involved in motility. Together, our results indicate that CsiA has a repressive effect on sporulation, but does not have an apparent impact on toxin and biofilm production or motility.

Conflict of interest: No

P 65

Structural characterisation of the *Clostridium difficile* S-layer

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Clostridium difficile cell surface is coated with a paracrystalline surface layer (S-layer), proposed to facilitate interactions with host cells (1). The main components of the *C. difficile* S-layer are two surface layer proteins (SLPs) derived from post-translational cleavage of a single precursor, SlpA: a high-molecular-weight protein (~40 kDa, HMW SLP) and a low-molecular-weight protein (~35kDa, LMW SLP). The two proteins interact to form a tightly associated non-covalent heterodimeric complex (H/L), (2).

Here we report the crystal structure of recombinantly produced interacting domains (HID/LID) of the H/L complex of *C. difficile*. The structural data led to identification of the interface residues of the SlpA heterodimer which were then probed using mutagenesis and biochemical assays. This structural and functional characterisation contributed to determining the structure of the native *C. difficile* S-layer and is allowing us to develop a model of the assembly. This work lays the foundations for potential structure-driven drug targeting of the mature S-layer in *C. difficile*.

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Conflict of interest: No

P 66

Functional analyses of the cell wall-associated fibronectin-binding proteins, FbpC and FbpD, in *Clostridium perfringens*

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Clostridium perfringens is a Gram-positive spore-forming anaerobe, causing gas gangrene and food poisoning for humans and animals. Human fibronectin (Fn) binds to the bacterial cells. We found two cell wall-associated Fn-binding proteins (56- and 45-kDa Fbps). These 56- and 45-kDa Fbps were named FbpC (CPE0625) and FbpD (CPE0630), respectively [1].
In this work, we attempted to elucidate the functions of FbpC and FbpD. The Fn-binding activity of *C. perfringens* HN13 Δ*fbpC* cells decreased compared to that of wild type strain, HN13. The complementation of fbpC gene restored the Fn-binding of the cells marginally. FbpC contains 12 putative cell wall-binding repeats (CW_binding_1) in the N-terminal region and a zinc carboxypeptidase (Peptidase_14) motif in the C-terminal region. The N-terminal region could stay in the peptidoglycan layers of the bacterial cells, and had the Fn-binding activity. On the other hand, the Fn-binding activity of HN13 Δ*fbpD* cells increased compared to that of HN13. Unexpectedly, the result of the complementation of fbpD gene suggested that FpbD might slightly enhance the Fn-binding to the cell. FbpD also conteins 10 putative cell wall-binding repeats (CW_binding_1) in the N-terminal region and a bacteriophage peptidoglycan hydrolase (Amidase_5) motif in the C-terminal region. Recent work showed that the recombinant FbpD degraded the cell walls by an enzymographic method.
In conclusion, FbpC and FbpD seem not to contribute as main Fn-binding proteins to enhance the Fn-binding to the *C. perfringens* cells although the proteins have strong Fn-binding activity themselves. It is possible that FbpC and FbpD might be involved in the growth and division of the *C. perfringens* cells naturally.

[1] Katayama, S., et al. 2015. Int J Anal Bio-Sci 3:1-9.

Conflict of interest: No

P 67

Fulminant *Clostridioides difficile* infection induces thymic atrophy and thymocyte death, in a toxin and time dependent manner

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Clostridioides difficile is the most common identifiable cause of infectious antibiotic-associated diarrhoea in developed countries, with disease mediated by the action of the two major toxins TcdA and TcdB, and a third toxin, CDT. In severe cases, disease can be fatal, with the patient presenting with severe colonic damage and, less frequently, with systemic disease complications. Systemic disease in animal models has also been described, with thymic disorganisation and apoptosis occurring during severe disease in mice. Here, we examine this disease phenotype and show that a ribotype 027 strain of *C. difficile* causes toxin-dependent thymic atrophy and disorganisation in mice. Disease damage results in reduced thymic size, disorganisation of the thymic cortex and medulla, as well as a reduction of the CD4+CD8+ thymocyte population and proportional changes in the single positive CD4+ or CD8+ naïve T cell populations. We also showed that thymic atrophy is not restricted to a single strain or genetic background of *C. difficile*, with strains from different ribotypes able to induce disease and thymic atrophy. This atrophy appeared to be linked to the severity of colonic damage, as thymic atrophy was absent in mice that were infected with strains that did not induce severe colonic damage. Administration of Bezlotoxumab, a monoclonal TcdB therapeutic produced by Merck, rescued mice from systemic disease, preventing thymic atrophy and CD4+CD8+ thymocyte depletion, as well as increasing mouse survival, when compared to mock treatment with PBS. These data suggest that the dissemination of gut contents, including toxins, into circulation as a result of enteric damage may influence systemic disease. As the thymus has such a crucial role in T cell production and immune system development, these findings may have important implications in relapse of *C. difficile* disease and impaired immunity during *C. difficile* infection.

Conflict of interest: No

P 68/FL 4

PPEP-1: a metalloprotease with multiple roles during *C. difficile* pathogenesis

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Clostridioides difficile is the most frequently reported nosocomial intestinal pathogen and is a major global public health concern. Colonisation of the gut epithelium is crucial in establishing a successful *C. difficile* infection. However, the molecular pathways and processes that modulate interactions between the bacteria and the host have been understudied. PPEP-1 is a secreted zinc metalloprotease which has been shown to cleave fibrinogen Bβ-chains and fibronectin, two components of the extracellular matrix, as well as CD2831, a *C. difficile* cell-surface adhesin. A PPEP-1 mutant also exhibited attenuated virulence in hamsters, highlighting a role for PPEP-1 in infection.
The aim of this project is to investigate the roles of PPEP-1 in *C. difficile* colonisation. As PPEP-1 is known to cleave bacterial cell surface adhesins, we first studied the role of this enzyme in modulating formation of biofilms, which are thought to be important in gut colonisation. We demonstrated that a PPEP-1 mutant forms significantly more biofilm than the wildtype (WT) *in vitro*. Genetic complementation with PPEP-1 restored biofilm formation to WT levels. To study the functions of this enzyme during *C. difficile* infection of the gut epithelium, we compared adhesion of the PPEP-1 mutant and the WT to intestinal epithelial cells in an in vitro dual environment 3D human gut model. The number of bacteria adhered to the epithelial cell layer were quantified using CFU counts and confocal microscopy. Our data suggest that the PPEP-1 mutant exhibits an enhanced adherence to the gut epithelium compared to the WT. Interestingly, although there appears to be more adhesion with the PPEP-1 mutant, the epithelial layer is less damaged as compared to the WT. Additionally, we see interesting differences in actin reorganisation in cell layers infected by the PPEP-1 mutant. We are currently investigating how PPEP-1 impacts bacterial penetration to underlying gut cell layers via degradation of the host extracellular matrix proteins. Our results indicate that PPEP-1 plays a key role in the modulation *C. difficile* biofilm formation and attachment to the gut mucosa, as well as a potential role in facilitating the penetration of the epithelial cell layer.

Conflict of interest: No

P 69

Reptiles are relevant carriers of *Clostridioides difficile*

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Clostridioides (previously *Clostridium*) *difficile* can cause human gastrointestinal infections ranging from mild diarrhoea to life-threatening pseudomembranous colitis that are often healthcare associated. Nevertheless, one quarter of the human *C. difficile* infections (CDI) is estimated to occur within the community (1) and a high overlap of ribotypes occurring in humans and various animal species has already been described (2) pointing to possible zoonotic transmission. Reptiles are increasingly popular pets and there is virtually no information about their *C. difficile* carriage and burden of disease.
During November 2017 and March 2019, 48 faecal (n=13) and organ samples (n=35) were collected originating from 25 individuals belonging to 16 different reptile species of different origin. All samples were screened for the presence of *C. difficile* by direct plating and enrichment culturing according to A. Schneeberg et al. (3). All *C. difficile* strains will be characterized using capillary gel electrophoresis based PCR ribotyping and PCR detection of toxin genes A, B, and the binary toxin genes. Additionally, Multilocus VNTR Analysis (MLVA) and antimicrobial susceptibility testing will be performed.
C. difficile was isolated from 15 (29%) out of 51 samples originating from 12/25 (48%) tested individuals. Although molecular characterization has not yet been completed, preliminary results revealed some well known toxigenic ribotypes that have already previously been related to livestock, humans or the environment, e.g. ribotypes 002/2, 005, and 049. Clinical and pathological data suggest that *C. difficile* can be associated with fatal conditions in reptiles.
This is the first survey on *C. difficile* in various reptile species. The results presented here prove that isolation rates of *C. difficile* in reptiles are comparably high; hence, *C. difficile* has to be considered as a potential pathogen in reptiles. Furthermore, reptiles may represent a possible source of infection for CDI in humans.

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Conflict of interest: No

P 70

Peptidoglycan remodelling throughout early engulfment in *Clostridium difficile* sporulation

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Clostridium difficile is the leading cause of healthcare associated diarrhoea. As an obligate anaerobe *C. difficile* is transmitted through the aerobic environment between hosts as a dormant cell type, the endospore. During sporulation the mother cell divides asymmetrically designating part of the cytoplasm as the forespore. The forespore is later engulfed by the mother cell, in a process requiring the coordinated synthesis and degradation of peptidoglycan (PG) (1). The precise mechanisms of this
process, and their regulation, remain largely unknown.
Here, we characterised PG variation throughout engulfment by combining strains lacking either cell-specific sigma factors that regulate sporulation, or PG degradation enzymes with mass spectrometry. We demonstrated that SpoIID-SpoIIP hydrolases work together to degrade PG, and deletion of either
cognate gene prevents engulfment. We hypothesised that PG deacetylases are involved in a mechanism that ensures only PG at the advancing edge is degraded during *C. difficile* engulfment,
thus preventing premature mother cell lysis, and have been investigating several of these putative enzymes.

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Conflict of interest: No

P 71

Characteristics of rifaximin resistance according to the PCR ribotype of *Clostridioides difficile*

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Clostridioides difficile is the etiological agent of antibiotic-associated disease and the most common cause of hospital-acquired infectious diarrhea. Rifaximin (RFX) has been proposed as an alternative treatment for specific cases of *C. difficile* infection (CDI) due to anti-inflammatory and antibacterial properties and additionally, poor absorption when taken by mouth. RFX-resistant *C. difficile* has occasionally been reported. We studied the RFX resistance and related resistance mechanisms in recent *C. difficile* isolates based on PCR ribotype.
In total, 413 unduplicated clinical isolates of *C. difficile* recovered from patients suspected with CDI at 2 tertiary hospitals in 2018 were tested. PCR ribotyping was performed using the primers 5′-CTGGGGTGAAGTCGTAACAAGG-3′ (nt 1445–1466 of the 16S rRNA gene) and 5′-GCGCCCTTTGTAGCTTGACC-3′ (nt 20–1 of the 23S rRNA gene) (1). MICs of RFX were determined using CLSI agar dilution methods. RFX susceptibility was categorized according to MICs (≤0.015 mg/mL, 0.03-64 mg/mL, and ≥128 mg/mL). Although CLSI breakpoints for C. diffcile are not defined for RFX, these categories are used to refer to isolates as RFX susceptible, RFX intermediate, and RFX resistant, respectively. To identify the *rpoB* mutations in strains with non-susceptible to RFX, PCR amplification using primers CDrpoB2-F (5’-ATGGAAGCTATAACGCCTCAA-3’) and CDrpoB2-R (5’-ACAGCACCATTTACAGTTCTA-3’), and sequencing of the rpoB gene was performed (2).
Overall resistance rate to RFX was 16.7% and there was no difference between the two hospitals. No rpoB mutation was observed in strains with RFX MIC ≤ 0.03 (336, 81.4%). Resistance to RFX was different for each ribotype. Among 48 identified PCR ribotypes, R027 (5/5,100%), R017 (12/21, 57%) and R018 (46/90, 51%) showed high resistance rate to RFX. A few isolate of R014/020, R012, R002, R046 and R159 showed resistance to RFX. There were 10 different RpoB polymorphisms identifed in the RFX-intermediate and –resistant *C. difficile* isolates. The combination of R505K/I548M and H502N/R505K amino acid substitutions were 73.3% of RpoB mutation. The 6 RFX-intermediate isolates

contained the single amino acid substitution of H502N, S498T, and S550Y. The majority of these amino acid substitutions have been previously reported (2,3). However, the combination of L487S/R505K and D492E/R505K amino acid substitutions are both novel in my knowledge.

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Conflict of interest: No

P 72

Metabolic crosstalk between *Clostridioides difficile* and human epithelial cells

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The intestinal pathogen *Clostridioides difficile* has attracted widespread attention as the most common and severe cause of antibiotic-associated diarrhea. Antibiotics alter the microbial and metabolic composition of the intestinal tract and *C. difficile* is capable of exploiting the newly created nutritional environment for germination and rapid growth. Consequently, depleted nutrient levels signal the secretion of toxins, which are internalized into epithelial cells and cause cellular and structural damage to the epithelium. Currently, research on toxin-independent interactions between *C. difficile* and host cells is limited.
Here, we analyzed metabolic host-pathogen interactions by the use of an in vitro co-cultivation system. In this study we used the epithelial cancer cell line HT29 and *C. difficile* strain 630Δerm. We were able to prove that both organisms can be cultivated in the same medium at hypoxic conditions, while being separated by a microporeous membrane. We analyzed the intra- and extracellular metabolome of HT29 cells via gas chromatography coupled to mass spectromery (GC-MS).
We observed that the co-cultivation with *C. difficile* in a transwell system induced strong shifts in the metabolome of HT29 cells. Already 2 hours after infection, the intracellular levels of the TCA cycle intermediate succinate increased. Succinate, a key player of cellular metabolism exhibits far-reaching effects on inflammation and hypoxia-related cellular physiology.
Concluding, the co-cultivation of mammalian epithelial cells with *C. difficile* leads to dramatic metabolic alterations in the HT29 cells. This clearly indicates that host-pathogen interactions during *C. difficile* infection are not only modulated by *C. difficile* toxins.

Conflict of interest: No

P 73

Structural and functional insight into the cell wall proteins from *Clostridium difficile*

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C. difficile is a leading cause of antibiotic-associated diarrhea that can lead to life-threatening complications (1). While cell surface proteins play an important role in the colonization, which is the first required stage of the infection that is followed by disease-causing toxin production, scarce structural data limit our understanding of their interactions within the bacterial cell wall and with their environment. Recently, the crystal structures of multi-domain cell wall proteins (CWPs) Cwp8 and CwP 6 from *C. difficile* revealed the cell wall binding type 2 (CWB2) module (2) that is shared among 29 CWPs of *C. difficile* 630 and represents one of the two evolutionary conserved surface layer (S-layer)-anchoring modules in Gram-positive bacteria. The aim of our studies is to gain structural insight into the CWPs to understand the functions of these proteins acting either alone or as a part of the S-layer assembly.
The mature CwP 5 (i.e. without the signal sequence) from *C. difficile* 630 was overexpressed in *Escherichia coli* BL21(DE3), purified by Ni-affinity and size exclusion chromatography. After spontaneous degradation of CwP 5 the resulting C-terminal fragment was identified by mass spectrometry and crystallized by sitting-drop vapor-diffusion technique using optimised commercial screens. Platinum derivatives of the crystals were prepared by soaking. Single-wavelength anomalous dispersion method revealed a two-faced right-handed β-helix crystal structure of the functional domain of CwP 5. Site-directed mutagenesis showed that CwP 5 undergoes intramolecular autoproteolysis, most likely similar to the maturation mechanism of CwpV (3), indicating a possible common autoprocessing mechanism of *C. difficile* CWPs and other CWB2 module-containing S-layer proteins of Gram-positive bacteria.

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Conflict of interest: No

P 74

Effect of sub-inhibitory concentrations of metronidazole on morphology, mobility, biofilm formation and metabolism of *Clostridium difficile*

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Clostridium difficile (CD) is responsible for various intestinal symptoms from mild diarrhea to severe pseudomembranous colitis and is the first cause of antibiotic-associated diarrhea in adult. *C. difficile* infection (CDI) treatment requires mostly specific antibiotics such as metronidazole, vancomycin or fidaxomycin. However, approximately 20% of treated patients experience recurrences. Its treatment is complicated by decreased susceptibility to antibiotics. Recent studies have indeed reported the increase of *C. difficile* strains with reduced susceptibility to metronidazole (MTZ). However, the mechanism of resistance remains unclear. Here, the impact of MTZ on morphology, mobility, biofilm formation and metabolism in two *C. difficile* strains VPI 10463 and CD17-146, showing different susceptibility profiles to MTZ was investigated. These two *C. difficile* strains were grown in BHI-SG with and without sub-inhibitory concentrations of MTZ. The biofilm production in vitro was quantified by crystal violet binding assay and by enumeration of viable cells and spores. Bacterial mobility in presence or absence of MTZ was studied in semi-solid agar. Surface proteins extracted from cell wall of bacteria cultivated with and without sub-inhibitory concentration of MTZ have been identified by mass spectrometry, allowing proteomic differential analysis. Our results revealed that sub-inhibitory concentrations of MTZ induced for the two strains diminished mobility and alteration of protein expression involved in biofilm production, protein biosynthesis, glycolysis, nitrosative stress as well as in DNA repair. Elongation morphology and enhanced biofilm production were only observed with the strain CD17-146, exhibiting a reduced susceptibility to this antibiotic.

Conflict of interest: No

P 75

Towards structure elucidation of the proline reductase complex from *Clostridioides difficile*

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"Stickland fermentation" is exclusively found in proteolytic Clostridia, where it enables these bacteria to use amino acids as a source of carbon and energy. Several studies unveiled clear links between proline uptake and Stickland fermentation to the three major complications associated with Clostridioides infections, namely its growth, sporulation and toxin production (1, 2, 3). This emphasizes the need for detailed knowledge about this central metabolic pathway. However, with the exception of general biochemical characterization, pertinent studies for the fundamental understanding of the Stickland fermentation are still underrepresented. The present study aims at filling this gap by addressing the Proline Reductase Complex (PRC) in terms of its structural biology.
The selenocysteine-containing PRC is encoded by the two genes prdA and prdB. The subunits assemble into an 800 kDa complex that is most likely composed of an octamer of PrdA/B-heterodimers. In this complex, PrdA undergoes seleno-cysteineolysis to give two fragments that are essential for generation of the substrate D-proline binding site, whereas PrdB complements the active site with a highly reactive selenocysteine/cysteine diad.
First crystallization experiments were not successful, which may be a consequence of the large molecular weight coupled to intrinsic flexibility, motivating us to address structure elucidation by Transmission Electron Microscopy (TEM) and cryo-Electron Microscopy (cryoEM). First results of negative staining TEM unveiled a complex overall shape of almost 20 nm diameter, in line with previous observations.
Further investigations are directed to the isolated 25 kDa subunit PrdB, which could not be crystallized but revealed suitable properties for structure determination via Nuclear Magnetic Resonance Spectroscopy (NMR). The combination of structural methods such as EM and NMR together with a set of other biophysical

experiments will improve our detailed understanding of the central metabolic player PRC and may hopefully generate new starting points for the alleviation of *C. difficile* infections.

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Conflict of interest: No

P 76

Towards phage therapy against *Clostridioides difficile*

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Clostridioides difficile is a serious public health threat, imposing a considerable burden on health services worldwide. Treatment of *C. difficile* infection (CDI) has become a rising problem due to the increasing occurrence of hypervirulent strains, antibiotic resistance and high frequency of recurrence. The rise of CDI epidemics over the last few years has triggered an increased interest in using phages as a form of therapy. Natural phage therapies rely on the lytic activity of phages to eradicate infection. To this date, no strictly lytic phage infecting *C. difficile* has been isolated. To address this issue, we have focused on the development of temperate synthetic phage infecting *C. difficile*.
We propose to test the concept of using temperate phage to deliver lethal cargo to *C. difficile* in the form of CRISPR/Cas9 arrays, directed at toxic genes specific to this pathogen. Once expressed in the cell, Cas9 directed towards a gene of interest would induce DNA double strand breaks. Since an efficient, non-homologous end-joining (NHEJ) or functional recombineering system is not available in *C. difficile*, Cas9 eventually degrades all the DNA, killing the bacterium.
Towards our strategy of combating *C. difficile* with phage therapy we have isolated phages Φ3801, Φ8601 and Φ9301 with broad-spectrum activity for PCR ribotype 078 strains. Phages have been identified and the genome sequence has been determined. Temperate phages, can feasibly be engineered when inserted in the bacterial chromosome using the same strategies developed for the bacterial host. The CRISPR/Cas9 genome editing tool was chosen to generate *C. difficile* manufacturing strains with deleted toxin genes and lysogens of these strains were made to enable genome editing of the phage DNA. Implementation of gene knock-ins in the phage genomes has begun, with the allelic coupled exchange strategy being used to deliver cargo into the phage. Cargo containing antibiotic selection markers have been used to test several potential locations in phage genome for gene insertion into the holin region of Φ9301 prophage genome.
In conclusion, several locations have been identified and experimentally proven for gene knock-in using antibiotic marker. Further work is required to determine

whether the Cas9 gene can be inserted into these locations and packed into functional viral particles.

Conflict of interest: No

P 77

Conditional gene essentiality in an S-layer deficient strain of *Clostridium difficile*

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On the *C. difficile* cell surface is a proteinaceous paracrystalline array, known as the S-layer. The S-layer of *C. difficile* is composed of two proteins: the high molecular weight (HMW) and the low molecular weight (LMW) S-layer proteins, derived from the pre-protein SlpA. Previous efforts to knock out slpA have proved unsuccessful. 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, we now have the opportunity to examine which genes become conditionally essential or non-essential in an S-layer deficient strain. Transposon directed insertion site sequencing (TraDIS) combines high-density transposon mutagenesis with high-throughput sequencing, allowing essential genes to be identified. This technique was previously applied to *C. difficile* strain R20291, allowing the identification of 404 genes required for growth and 798 for sporulation in vitro. We are now extending this analysis to an S-layer null strain to identify changes in gene essentiality. To allow analysis of gene function in the wild type strain and S-layer deficient strain, we have also constructed a CRISPR interference (CRISPRi) system in *C. difficile* which will allow analysis of gene essentiality and also gene function during transient knock-down.

Conflict of interest: No

P 78

Development of a novel method for efficient and precise genome editing with *mazF* in *Clostridium perfringens*

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Clostridium perfringens is one of the fastest growing bacteria, with doubling times less than 10 min under optimum growth conditions. The organism produces potent histolytic enzymes and toxins, causing various diseases ranging from mild food-borne diarrhea to fulminant histotoxic infection. Other clostridial species also produce various toxins and useful enzymes, but their applications have been limited to those with relatively low molecular weight, probably due to difficulty of expression using existing expression systems caused by their distinct preferences in codon usage. To facilitate genome-wide identification of virulence factors and biotechnological application of their useful products, we have previously established the GalK/galactose-based counter-selection system for a precise genome editing in *C. perfringens*[1]. However, to employ this system, the *galKT* operon must be disrupted, resulted in unable to metabolize galactose, which might affect the pathogenicity and the productivity of substances. In order to overcome the disadvantage, we developed a novel markerless genome-editing method for *C. perfringens* which allows multiple-gene deletion and substitution. This method employed *Escherichia coli* MazF-toxin gene as the counter-selectable marker under the control of the xylose-inducible ON/OFF gene expression system on a suicide plasmid, pXM. It was shown to be accurate and simple to disrupt the alpha-toxin gene on the chromosome of the organism. We also succeeded in allelic exchange of theta-toxin gene, *pfoA* with *eglA* gene from *C. acetobutylicum* and expression of *eglA* under the control of *pfoA* promoter. These results suggest that this novel system has many advantages in studies for virulence factors to pathogenesis and for production of useful substances based on metabolic engineering.

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This work was supported by Chubu University Grant (A).

Conflict of interest: No

P 79

Role of *pdeB* gene in sporulation initiation of *Clostridioides difficile*

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Clostridioides difficile is the number one cause of hospital-acquired infections in North America and many European countries (1). *C. difficile* infection (CDI) occurs as a consequence of antibiotic treatment which disrupts not only the pathogenic microorganisms but also the commensal gut microbiome, thus providing a favorable environment for an antibiotic-resistant pathogen like C.difficile to colonize and cause infection. C.difficile vegetative cell produces two enterotoxins A and B which cause life-threatening inflammation of the colon and severe diarrhea. When a *C. difficile* vegetative cell encounters any unfavorable environmental conditions like lack of nutrition, aerobic environment, host immune response, etc., it initiates the sporulation process and produces metabolically dormant spores. These spores are found abundantly in the hospital, and health care settings and are ready to be picked by the susceptible individual. Once *C. difficile* spores are ingested, they germinate in the large intestine to become active vegetative cells which produce toxins and cause inflammation and diarrheal symptoms. *C. difficile* spores are passed in the feces of the infected individual and are the primary agent for the transmission of the disease (2). One way to limit the spread of the disease is to inhibit spore formation. However, the specific environmental signal that triggers sporulation and gene regulatory network that regulates sporulation in *C. difficile* is poorly understood. This project proposes to understand the role of the pdeB gene, coding for a phosphodiesterase, in sporulation initiation of *C. difficile*. We created a mutant UK::*pdeB* and carried out sporulation assay to find that UK::*pdeB* strain was hypo sporulating and possessed higher intracellular c-di-GMP than the UK WT strain. We are currently exploring the role of c-di-GMP in sporulation initiation of *C. difficile*.

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Conflict of interest: No

P 80/FL 6

SecA2 and the Biogenesis of the *Clostridium difficile* S-layer

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Clostridium difficile cells are coated by a paracrystalline, proteinaceous array called the S-layer. The S-layer of *C. difficile* is mostly composed of two proteins created by the cleavage of the preprotein SlpA. Although SlpA constitutes approximately 15 % of the total protein content of the cell, its functions and the biogenesis of the S-layer remain poorly understood.
SlpA is secreted through the accessory Sec secretion system. The Sec pathway is composed of a channel through which proteins are translocated, SecYEG, and a motor protein, SecA. The Accessory Sec pathway is characterised by an additional, slightly smaller, SecA motor protein: SecA2. SecA2 is responsible for the secretion of SlpA and at least one other SlpA paralogue.
SecA2 pull-down experiments identified several potential SecA2-interacting partners, including potential secretion substrates and proteins involved in cell wall synthesis. Microscopy analysis of S-layer secretion reveals septum localisation. In addition, the MreB perturbing compound, A22, adversely affects SlpA secretion. Split-luciferase assays revealed an interaction between SecA2, FtsZ and MreB2.
Taken together, these data support the hypothesis that *C. difficile* S-layer biogenesis co-localises with cell wall synthesis. This will ensure the synchronised growth of the cell wall and S-layer, preventing the appearance of gaps in the S-layer at areas of cell wall growth.

Conflict of interest: No

P 81

Self-assembly of spore coats- emerging principles

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Bacterial spores are uniquely stable cell forms, highly resistant to harsh environmental insults. Spores have a robust protective coat, and in some cases an outer exosporium, made of many dozens of proteins. We have been addressing the question of how these multiple protein components are assembled into such robust architectures. Remarkably, a number of common principles of assembly are emerging in all the spores we have studied- *Bacillus anthracis/cereus/thruringiensis* (1,2), *Bacillus subtilis* (3), *Clostridium sporogenes* (in preparation) and *Clostridium difficile*. Coat and exosporium proteins expressed in E. coli can arrange intracellularly into highly stable macro-structures through processes of self-assembly. These proteins assemble to generate ordered one-dimensional fibres, two-dimensional sheets and three-dimensional stacks. In several cases, the high degree of order favours strong, cooperative intracellular disulphide cross-linking. We have applied this approach of heterologous expression to proteins from both *B. cereus* and *C. sporogenes* exosporium proteins. These proteins show a similar tendency for self-organization and in these cases we have been able to recapitulate structures seen in the native exosporium. Self-assembly of this kind could form exquisitely adapted building blocks for robust and cooperative higher-order assembly across all spore-formers.

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Conflict of interest: No

P 82

High resolution imaging of *Clostridium sporogenes* germination

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Clostridium botulinum is a Gram-positive, spore forming anaerobic bacteria that produces a lethal neurotoxin causing botulism. The lethality of *C. botulinum* and its ability to germinate within the human intestines means it poses a significant concern for the food industry and is considered to be a bioterrorism threat. Due to the risk level posed by *C. botulinum* it is rarely used in the laboratory. Instead *Clostridium* sporogenes is used as a surrogate due to its close similarity both physiologically and phylogenetically to *C. botulinum.* Spores are highly resistant to physical and chemical threats and so can thrive in environments that vegetative cells would not, allowing them to persist in the environment for prolonged periods of time. Germination of spores is a key process in which the dormant spores detect the presence of specific germinant molecules in the environment and go from being highly resistant, dormant spores to being vegetative cells. Despite being of significant importance, the process of germination in *C. sporogenes* is relatively poorly understood both morphologically and biochemically. A variety of amino acids are capable of triggering germination of *C. sporogenes* and, in other species, we are beginning to understand the biochemical details of spore core rehydration during the first stages of germination, seen physically as a change from phase bright to phase dark under a phase contrast microscope. Previous work from our laboratory has determined the structure of the *C. sporogenes* exosporium providing a strong basis for further structural studies. Using a range of microscopy techniques, including phase contrast microscopy, transmission electron microscopy (TEM) and atomic force microscopy, we have been able to track the morphological changes that occur throughout the germination process. By using phase contrast live cell imaging we can see in real-time the events that occur during germination and, from this, pick time points for TEM on fixed samples. This allows us to see at high resolution the morphological and structural differences of the germinating C. sporogenes spores. Progress is also being made in looking at knockout strains of C. sporogenes to see the effect of specific proteins on the germination process.

Conflict of interest: No

P 83

Reactive oxygen species in HEp-2 cells treated with *Clostridioides difficile* TcdB

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The two homologous toxins TcdA and TcdB are the main pathogenicity factors of *C. difficile*. Both toxins induce cell rounding by glucosylation of Rho GTPases. Specifically TcdB induces early cytotoxic effects on host cells which is triggered by reactive oxygen species (ROS). Inhibition of the NADPH oxidase prevents ROS-induced early cell death and allows cells to become apoptotic, instead. The molecular mechanism by which TcdB triggers cytotoxic ROS concentrations is not known. To elucidate TcdB-induced early cell death we investigated the production and metabolism of ROS in TcdB treated cells.
The application of specific for NADPH oxidase inhibitor and ROS scavenger such as apocynin reduced early cell death induced by TcdB, indicating an essential role in pathogenicity. In contrast, Inhibition of the xanthine oxidase by allopurinol had no effect on early cell death, speaking against significant mitochondrial damage within the early phase of intoxication. Surprisingly, we did not measure an overall increase in ROS production after treatment of cells with TcdB. Instead, we measured a concentration-dependent decrease in the activity of the NADPH oxidase. Since we measured no increase in ROS production, we investigated the metabolism of ROS in toxin treated cells. We found that the TcdB-induced early cell death was associated with reduced GSH content in cells. But previous depletion of GSH by buthionine sulfoximine in cells did not increase cytotoxic effect. Thus, reduced GSH is rather a sequel than the reason for loss of cell viability. The activity of the superoxide dismutase I and II was unaffected in toxin treated cells. As most promising target structure we found the catalase activity reduced in cells treated with TcdB. Although a direct inhibition of the catalase by TcdB was excluded, we found that additional inhibition of the catalase by 3-amino-1,2,4-triazol significantly increased cell death. In summary, our findings do not support the hypothesis of a TcdB-induced increase in ROS production by the NADPH oxidase. The decreased GSH level in TcdB treated cells indicates anti-oxidative activities of the glutathione system in cells where the catalase failed to metabolize radicals. Thus, TcdB rather affects metabolism than generation of ROS.

Conflict of interest: No

P 84

Structural characterisation of the *Clostridium sporogenes* exosporium

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Botulism is a severe and life-threatening disease caused by the release of toxins from *Clostridium botulinum*. Due to the lethality of the disease *C. botulinum* has been classified as a potential bioterrorism threat.
This obligate anaerobe forms endospores, a cell form that is able to withstand extreme environments. Endospores can remain in this metabolically dormant state for years and upon encountering germinants they can germinate within minutes. The endospore state is the infectious particle for the transmission and persistence of the infection.
The first point of contact between the pathogens and their host is its outermost layer, the exosporium. This proteinaceous layer is present across Bacilli and Clostridia and is variously involved in host cell targeting, germination, adherence and protection. Most structural work on exosporium has been conducted on the *Bacillus cereus* group- the structure of exosporium in the Clostridia group remains poorly understood.
We have been characterising the structure and composition of exosporium in *C. sporogenes,* an experimental surrogate for group 1 *C. botulinum*. We will present methods used to reveal the 3D structure of the exosporium in molecular detail; these include a combination of electron crystallography, atomic force microscopy and electron tomography. These give insights into the robustness and protective role of proteinaceous layers of Clostridial spores.

Conflict of interest: No

P 85/FL 5

Isolation of novel bacteriocins active against clinically relevant, pathogenic *Clostridioides difficile*

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The emergence of antibiotic resistant, hypervirulent strains of the nosocomial pathogen *Clostridioides difficile*, coupled with increased concerns over the physiological impact of traditional broad-spectrum antibiotics on human health has led to an increased demand for alternative therapies. Bacteriocins are proteinaceous toxins produced by bacteria that kill other bacteria and their antimicrobial activity makes them an attractive potential alternative therapy to antibiotics. This research aims to isolate environmental bacteria from porcine faeces that produce novel bacteriocins with inhibitory activity against *C. difficile*. At present in this study three distinct organisms with inhibitory activity against *C. difficile* have been isolated and it has been shown that these strains encode various bacteriocins. The current focus of this research aims to attribute the inhibitory activity to one of the encoded bacteriocins or potentially a novel antimicrobial peptide, which could then be exploited as an alternative therapy against *C. difficile*.

Conflict of interest: No

P 86

Determining the in situ structure of the *Clostridium difficile* envelope using combined electron microscopy techniques

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Many bacteria are coated with a proteinaceous outer surface layer (S-layer) implicated in structural integrity, localisation of enzymes to the surface of the bacteria, and in pathogenic organisms, interaction with the host (1). The *C. difficile* S-layer is made up of a paracrystalline layer of self-assembling SlpA, a heterodimeric protein complex made up of a high molecular weight (HMW) cell wall interacting subunit, and a low molecular weight (LMW) crystallisation subunit (2). 28 other *C. difficile* cell wall proteins (CWPs) contain the cell wall binding domain seen in the SlpA HMW , and it is thought these proteins will decorate the S-layer (3). Using 2D electron crystallography of intact C .difficile S-layers, we have been able to reconstruct the 3D structure of this layer, in both strain 630 and the clinically relevant R20291. This has allowed the recently determined X-ray structures of SlpA to be modelled into the in situ 3D reconstruction, to build a native structure of the *C. difficile* S-layer. Further, electron cryotomography has suggested that the other CWPs may form complex domains both on the surface, and underneath the S-layer.

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Conflict of interest: No

P 87

Investigation of *Clostridium difficile* toxin production under various in vitro growth conditions

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Clostridium difficile is a Gram-positive, spore-forming and anaerobic bacterium that is the most common cause of healthcare-associated diarrhea. Disease typically occurs when the normal intestinal colonic flora is disrupted by therapy with antibiotics or antitumor agents, and ingested *C. difficile* spores germinate in the colon. The vegetative cells produce and secrete the two toxins, TcdA and TcdB, usually causing diarrhea, which may lead to pseudomembranous colitis and infections are often fatal. The optimal growth conditions and toxin expression for *C. difficile* have been thoroughly studied. For instance, the concentration and type of sugars in the growth media have been shown to play a crucial role. Lately, the presence of trehalose was to shown to increase the disease severity of certain hypervirulent strains. We have conducted a systematic study to elucidate growth and toxin production in the presence of different sugars in comparison to trehalose. Furthermore, it was recently shown that *C. difficile* is able to grow under aerobic, normally toxic, conditions in the presence of *Candida albicans*. We have studied if presence of *C. albicans* influenced toxin production and whether aerobic growth of *C. difficile* is correlated to specific species. Our latest results will be presented at the meeting.

Conflict of interest: No

P 88

Discovering the metabolic aspects of host-pathogen interactions during *Clostridioides difficile* infection.

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Toxin A (TcdA) and Toxin B (TcdB) are the major pathogenic factors released by *Clostridioides difficile* during infection. These toxins disrupt intestinal epithelial barrier integrity, which leads in addition to its canonical targets to mucosal inflammation and in some cases colitis. Moreover, TcdB has been suggested to influence cellular metabolism of the host. It has been shown that TcdB causes a strong autophagic effect including intervention in mTOR signaling. Given that the immunological response requires changes in cellular metabolism, especially in mitochondrial metabolism, we sought to investigate the effect of TcdB on the metabolism of human macrophages.

We differentiated human macrophages from purified primary CD14 blood monocytes using M-CSF and then exposed them to TcdB with or without prior stimulation with a toll like receptor stimulus. As TcdB has also been associated with inflammasome activation, we inhibited caspase-1 to ensure that the effects observed were upstream of cell death. We then profiled the macrophage metabolism to investigate effects on the metabolic fluxes and the metabolome using 13C labeled glucose and glutamine.

Our study revealed that TcdB induced profound alterations of macrophage metabolism, including alterations in glycolysis, and changes in the flux of metabolites through the citric acid cycle. Intriguingly, TcdB also appeared to be able to reverse the effects of metabolic changes induced by toll like receptor stimulation. These effects may impair the ability of the macrophages to enact a full inflammatory response. Taken together our results suggest that the effect of TcdB reaches beyond disruption of the cytoskeleton and reveals potential targets for prevention of *C. difficile* induced colonic tissue damage.

Conflict of interest: No

P 89/FL 7

Clostridium difficile RT078 strains can utilize starches that are prevalent in animal feed and the human diet

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Clostridium difficile is the most common cause of antibiotic associated diarrhea and has become one of the most frequent hospital acquired infections in the US and Europe. In the early 2000's, there was a world-wide outbreak of *Clostridium difficile* infection (CDI) associated with increased morbidity and mortality. Ribotype 078 (RT078) strains emerged as epidemic strains in 2003 and are now a common *C. difficile* ribotype in hospitals and community-acquired outbreaks. Infections due to RT078 strains in humans were rare prior to 2000 and the reasons for increased infections in humans remains poorly understood. Interestingly, RT078 are amongst the most abundant *C. difficile* ribotype found in livestock and direct transmission between animals and humans has been described. We recently found that RT078 strains can utilize the sugar trehalose at lower concentrations most of the *C. difficile* ribotypes due to the acquisition of a genetic locus that contains genes necessary for the metabolism of trehalose. Interestingly, we find that RT078 strains also have the ability to grow on complex oligosaccharides and this ability is dependent on the *ptsT* gene, which encodes a phosphotransferase system transporter. We have demonstrated that expression of PtsT confers the ability of CD630 (RT012) to grow on low concentrations of trehalose and contributes to growth on the polysaccharides inulin and arabinogalactan in PtsT dependent manner. We have further tested the ability of a variety of *C. difficile* ribotypes to grow on starch substrates as a sole carbon source, and thus far found that only RT078 strains have the ability to utilize starch. We are currently investigating how the other genes in this operon may contribute to starch and polysaccharide utilization and how this metabolism impacts RT078 fitness and pathogenicity.

Conflict of interest: No

P 90

Diversity in *Clostridium botulinum* spore morphology

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Clostridium botulinum is a sporulating, gram-positive bacterium, ubiquitous in the environment. It produces the botulinum neurotoxin that causes botulism, a rare but extremely seriously paralytic disease. Spores play a central role in *C. botulinum* transmission and pathogenesis. Food-borne botulism is an intoxication where spores contaminate food products and may germinate and outgrow into toxic cultures upon food storage. Toxicoinfectious botulism follows when the spores present in the gut or in deep wounds germinate, colonize, and produce toxin in vivo.
C. botulinum is a diverse group of genetically and ecologically distinct clostridia. Group I strains are terrestrial, mesophilic, and produce highly heat-resistant spores. Thus they are frequently associated with food-borne botulism caused by canned vegetables, and with toxicoinfectious botulism where growth in mammalian body temperature is required. Group II strains can be terrestrial (type B) or aquatic (type E), and are generally psychrotrophic and produce spores of moderate heat resistance. They are mostly related to food-borne botulism due to refrigerated packaged seafood or cured meat, and hardly ever implicated with toxicoinfectious botulism. Group III strains are either terrestrial or aquatic, mesophilic or slightly thermophilic, and cause botulism in animals. Heat resistance properties of Group III spores are poorly understood.
Distinct physiology and ecology, and probably different natural niches for spore formation of different *C. botulinum* strains are likely factors to contribute to the spore composition and resistance properties. Here we examined the architectural and topological differences of spores from various *C. botulinum* Groups I, II, and III strains. Using transmission electron microscopy, we describe distinct spore structures and provide insights into respective biological properties and functions, such as heat resistance and adherence. This work contributes to clostridial spore biology and improves our understanding on how the morphological differences between *C. botulinum* spores can determine the spore properties.

Conflict of interest: No

P 91

The CspC pseudoprotease regulates germination of *Clostridioides difficile* spores in response to multiple environmental signals

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The gastrointestinal pathogen *Clostridioides difficile* initiates infection when the metabolically dormant spore form germinants in the small intestine. While most spore-forming bacteria studied to date use membrane-bound germinant receptors to sense nutrient germinants, *C. difficile* lacks homologs of these germinant receptors and instead senses specific mammalian bile salts to initiate germination. Genetic evidence has implicated the pseudoprotease, CspC, in directly sensing these bile salt germinants. To gain insight into *C. difficile*'s unique germination pathway, we solved the crystal structure of CspC from *C. difficile*. Guided by this structure, we generated mutations in CspC that rendered spores either hypo- or hypersensitive to bile salt germinant. Surprisingly, hypersensitive mutants also exhibited bile-salt independent germination and were more sensitive to amino acid and/or calcium co-germinants that potentiate spore germination in the presence of bile salts. As substitutions at individual amino acid residues in CspC changed the responsiveness of spores to both bile salt germinant and co-germinates, our work indicates that CspC integrates multiple environmental signals to induce germination and either directly or indirectly senses distinct classes of small molecules. Our work raises the possibility that CspC may not be a ligand binding receptor but may instead function as a signaling node.

Conflict of interest: No

P 92

Stool-derived inflammatory mediators serve as biomarkers of severity in *Clostridium difficile* infection

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Clostridium difficile infection (CDI) is a major public health concern and frequently results in severe disease, including death. Predicting subsequent complications early in the disease course can assist clinicians in their decision-making and potentially improve outcomes. However, models based on clinical criteria alone are not accurate and/or do not validate. We hypothesized that inflammatory mediators from the stool would be biomarkers for severity and complications.
Consecutive subjects with available stools specimens were included if they tested positive for toxigenic *C. difficile* from the clinical microbiology laboratory, only after formed stools were first rejected. Testing included two steps: enzyme immunoassay (EIA) for glutamate dehydrogenase and toxins A/B, with reflex to PCR for the *tcdB* gene for discordants. Stool was diluted 1:1 in a mixture of PBS and protease inhibitor, centrifuged, and the supernatant stored on ice until analysis. A 17-plex panel of inflammatory mediators was run on a Luminex 200 machine using a custom antibody-linked bead array. Prior to analysis, all measurements were normalized and log-transformed. Severity was defined by IDSA criteria (serum white blood cell count >15000 cells/microliter or creatinine 1.5 times the pre-morbid baseline). The complications studied were 30-day all-cause mortality and disease-related complications (DRC): ICU admission, colectomy, and/or death within 30 days due to CDI, as determined by physician adjudicators. Principal component and redundancy analyses were used to assess overall variation in the panel between outcomes, and significance testing was performed with a permutational, multivariate ANOVA (PERMANOVA). Initial bivariable analyses were performed by logistic regression, followed by machine learning modeling using elastic net regression with 5-fold cross validation. The area under the receiver operator characteristic curve (AuROC) was computed for the cross-validated models.
We included 225 subjects, with 124 females (55.1%) and average age 58.5 (+/-17). More patients were diagnosed by PCR than toxin EIA (170 vs. 55, respectively). IDSA severity, death, and DRCs occurred in 79 (35.1%), 14 (6.2%), and 12 (5.3%) subjects, respectively. PCA and PERMANOVA revealed that the panel overall associated with IDSA severity (P =.009) but not death or DRCs. On unadjusted

analysis, CXCL5, IL-8, and HGF associated with IDSA severity, EGF with death, and none with DRCs. Despite weak associations on standard linear analyses, the machine learning models performed well, resulting in AuROCs of 0.75 for IDSA severity, 0.84 for death, and 0.74 for DRCs.
We found that specific inflammatory mediators from the stool of patients with CDI can predict severity and complications. These results are promising, but need replication in a larger dataset and should be incorporated into models that include clinical covariates prior to clinical deployment.

Conflict of interest: No

P 93

Epigenetic regulation of *Clostridioides difficile* spore formation by a conserved orphan DNA methyltransferase

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Clostridioides difficile is a leading cause of antibiotic-associated diarrhea worldwide. Since *C. difficile*'s metabolically dormant spore form is the major infectious particle of this obligate anaerobe, spore formation is essential for *C. difficile* to survive exit from the host and transmit disease. Similar to other spore-forming organisms, *C. difficile* uses the master transcriptional activator, Spo0A, and four sporulation-specific sigma factors to control the transcriptional program that ultimately leads to the production of dormant spores. Notably, only a subset of *C. difficile* cells initiate spore formation during infection, and the mechanisms controlling this decision remain poorly characterized. Using mutational and RNA-Seq analyses, we have determined that an orphan DNA methyltransferase, CamA, enhances the number of spores produced by *C. difficile* by acting at a step downstream of Spo0A activation. qRT-PCR and fluorescence microscopy analyses using morphological stains and visualizable transcriptional reporters indicate that activation of the early-stage sporulation-specific sigma factor, SigF, is diminished in a *camA* mutant. We are currently conducting targeted mutational analyses to identify specific methylation sites that affect the frequency of SigF activation. Since the decreased spore formation observed in a *camA* mutant correlates with a reduced ability to persist in a mouse colonization model relative to wild type, our analyses implicate epigenetic regulation as a novel mechanism for controlling *C. difficile* disease transmission.

Conflict of interest: Yes

Disclosures: Consultant for diagnostic start-up

P 94

Impact of Orphan Phosphotransfer Proteins on *Clostridioides difficile* Sporulation

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The formation of spores is critical for survival of *Clostridium difficile* outside of the host gastrointestinal tract. Persistence of *C. difficile* spores greatly contributes to the spread of *C. difficile* infection (CDI), and the resistance of spores to antimicrobials facilitates the relapse of infection. The initiation of sporulation is regulated through activation of the conserved transcription factor, Spo0A, however the mechanism of Spo0A regulation is not understood. Multiple kinases and phosphatases regulate Spo0A activity in other species, but these factors are not well conserved in *C. difficile*. Previously, we found that deletion of the predicted orphan kinase, CD1492, results in increased sporulation, indicating that CD1492 is a negative regulator of sporulation. In this study, we investigate the functions of orphan phosphotransfer proteins, which were originally proposed to promote sporulation by directly phosphorylating Spo0A. We found that disruption of another orphan kinase, CD1949, had no apparent effect on sporulation. However, disruption of a third orphan kinase, CD2492, resulted in hypersporulation, comparable to the CD1492 null mutant and in contrast to a prior report. Moreover, a CD2492 CD1492 double mutant mirrored the sporulation phenotypes of the CD1492 and CD2492 mutants, suggesting that both of these phosphotransfer proteins repress sporulation. In addition to the sporulation phenotypes of the CD1492 and CD2492 mutants, both disruptions resulted in a decrease in toxin expression and *sigD* transcription. Together, these data suggest that CD1492 and CD2492 function in the same genetic pathway to regulate sporulation initiation. These results provide further evidence that *C. difficile* sporulation is regulated differently from that of other endospore forming species.

Conflict of interest: No

P 95

Validation of systemic inflammatory mediators as biomarkers for severity and adverse outcomes in *Clostridium difficile* infection

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Clostridium difficile infection (CDI) causes a range of disease severity from asymptomatic colonization to colitis and death. Current risk scores do not allow for the early prediction of death or adverse outcomes. We previously showed that multiple inflammatory mediators were associated with severity and adverse outcomes, and here we sought to validate these results.
Sera was collected within 48 hours of diagnosis of CDI in two distinct cohorts of patients. The pilot cohort collections ranged from October 2010 to November 2012 and the validation cohort collections ranged from January to September 2016. All patients were diagnosed with CDI by the clinical microbiology laboratory using a two-step algorithm including detection of *C. difficile* glutamate dehydrogenase (GDH) and toxins A/B by enzyme immunoassay, with reflex to PCR for tcdB gene for discordant results. Concentrations of select inflammatory mediators were measured using a custom multiplex assay on a Luminex 200 machine. Severity was defined by IDSA criteria (WBC > 15,000 cells/mL or creatinine > 1.5 mg/dL). The primary outcomes were 30-day all-cause mortality and a composite adverse outcome: ICU admission, colectomy or death within 30 days attributed to CDI. Principal components, redundancy analysis, and biplots were used to characterize inflammatory mediator profiles, with permutational, multivariate ANOVA (PERMANOVA) for significance testing between groups. Unadjusted and adjusted models were built using logistic and elastic net regression, respectively. Models were compared using area under the receiver operator characteristic curve (AUC).
The pilot cohort included 156 unique CDI cases, and 63 (40%) had severe CDI, 4 (3%) died, and 10 (6%) had the composite adverse outcome. In this pilot, RDA differentiated IDSA severe and non-severe episodes by PERMANOVA ($P = .01$), and the PCA axis 1 explained 30% of total variation. In unadjusted analysis, IL-2R, IL-6, and procalcitonin associated with severity ($P < 0.001$, $P = 0.003$, & $P = 0.003$

respectively). In our validation cohort, we included 272 unique CDI cases from 253 patients, and 71 (26%) had severe CDI, 19 (7%) died, and 18 (7%) had the composite adverse outcome. RDA differentiated IDSA severity, 30-day mortality, and composite adverse outcomes by PERMANOVA (P = 0.001, P = 0.001, and P = 0.002 respectively), with PCA axis 1 explaining 29.1% of total variation. Unadjusted analyses consistently identified HGF, IL-2R, procalcitonin, IL-8, and/or IL-6 as predictors of severity and outcomes. For IDSA severity, the 5-fold cross-validated elastic net model containing HGF and procalcitonin had an AUC of 0.74 [0.67-0.81]. For 30-Day mortality, a model containing IL−2R, IL−8, procalcitonin, IP−10, and CXCL-5 had an AUC of 0.89 [0.83-0.95]. Lastly, for the composite adverse outcome a model containing IL-8, procalcitonin, HGF, and IL-2R had an AUC of 0.84 [0.74-0.95].
This study validated the hypothesis that a panel of biomarkers from sera associated with severe CDI and predicted adverse outcomes. Specific inflammatory mediators performed the best, including HGF, IL-2Ra, procalcitonin, and IL-8. Our results support the possibility of a serum-based biomarker panel to inform medical decision making for patients with CDI.

Conflict of interest: No

P 96

The collagen-like exosporium BclA proteins affect the assembly of the outer layers and the biology of *Clostridioides difficile* spores

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Clostridioides difficile spores are considered the morphotype of infection, transmission and persistence of *C. difficile* infections. The outermost exosporium layer is thought to contribute to spore-host interactions early during infection and during recurrence of the disease. We have previously demonstrated that spores formed from the same isogenic culture can have a thick or a thin exosporium, nevertheless, both types of spores exhibit hair-like projections. The *C. difficile* spore exosporium proteins involved in the formation of these hair-like extensions and their implications in spore-biology and pathogenesis remains unclear. In this work, we used an epidemically relevant *C. difficile* strain, R20291, to assess the implications of the collagen-like BclA2 and BclA3 exosporium proteins in the formation of the hair-like extensions, spore biology and pathogenesis. Through transmission electron microscopy, we demonstrate how the absence of these BclA proteins affects the formation of the hair-like extensions as well as the exosporium layer of *C. difficile* spores. Immunogold labelling confirmed that the hair-like projections are formed by BclA2 and BclA3. Electron micrographs also demonstrated that absence of BclA2 and BclA3 impacted the formation of the spore-coat underlying the exosporium layer. Through immunofluorescence microscopy with anti-BclA2 and BclA3 antibodies we demonstrate that in the absence of BclA3, BclA2 becomes more accessible to antibodies, suggesting that BclA2 may be buried in the exosporium layer. By infecting monolayers of intestinal epithelial Caco-2 cells, we observed that absence of BclA proteins affected adherence of *C. difficile* spores to intestinal epithelial cells. Finally, in a mouse model of infection, our data demonstrates that absence of BclA proteins does not affect the initiation of the infection of the epidemically relevant *C. difficile* strain R20291. Collectively, these results provide new evidence of the role of BclA proteins in *C. difficile* spore assembly and their impact in the pathogenesis of the disease.

Conflict of interest: No

P 97

Clostridium botulinum neurotoxin production and sporulation are affected by nutrient availability

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Early research already provided strong evidence that botulinum neurotoxin (BoNT) production and sporulation of *C. botulinum* are depending largely on environmental and metabolic factors. Availability and type of carbon sources are critical determinants of BoNT production in *C. botulinum* cultures (1, 2). While glucose is required for efficient BoNT expression, arginine in high concentration represses both BoNT expression and sporulation (3). For gram-positive bacteria it has been generally accepted that cell metabolism and virulence are closely related, however, the underlying regulatory mechanisms are only partly understood.
We have identified several carbohydrates and amino acids as nutrient sources that have a concentration-dependent influence on BoNT production and sporulation in *C. botulinum* Group I cultures grown in poor medium. Whereas the presence of most tested carbohydrates at low concentration induced BoNT production and sporulation compared to the control medium, higher concentrations led to reduced BoNT concentrations and spore counts. This suppressive effect suggests a significant role of carbon catabolite repression in the regulation of *C. botulinum* pathogenicity when other nutrients are limited.
Increase in BoNT production and sporulation was detected after supplementation of control medium with amino acids that can be oxidized in Stickland fermentations with proline or glycine. Preliminary proteomic data showed activation of the proline reductase complex already at early growth stage whereas the glycine reductase complex was strongly activated during stationary growth, suggesting proline to be the preferred Stickland partner over glycine.
We aim to understand which intracellular metabolites and metabolic pathways contribute to BoNT production and how these processes are regulated. This will help to infer the molecular network regulating the pathogenicity of *C. botulinum* and contribute to the development of countermeasures to prevent botulism.

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Conflict of interest: No

P 98

Advanced modular vectors for research and development of Clostridia

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Clostridium is a medically and industrially important genus of bacteria. These obligate anaerobes are not easy to manipulate on the genetic level. Efficient plasmid transformation and genetic modification of strains from the genus *Clostridium* has become increasingly important for the study and exploitation of Clostridia for medical and industrial biotechnology. One rapidly growing area of interest is their role in the gut microbiome.
The Synthetic Biology Research Centre (SBRC), based at the University of Nottingham, has previously developed a suite of molecular biology tools that allows researchers to study and manipulate these important bacteria. This includes the modular pMTL80000 plasmids that support gene knockout, gene expression and chromosomal integration technologies including ClosTron, Allele Coupled Exchange (ACE), mariner transposon mutagenesis and recently CRISPR-Cas9. These tools have been demonstrated across a wide range of medically and industrially important species of *Clostridium*. Development of the vector series has led to the availability of six additional Gram-positive replicons, additional resistance markers and Gram-negative replicons. Palindromic restriction sites located in the plasmid sequence in a way to separate the modules allow easy and quick modification of all modules. Further, the addition of new modules that carry specific functions is not hindered by the need to create a new plasmid but can simply be designed to slot into the existing module. Developments of the original pMTL80000 series such as allelic exchange vectors, transposon vectors and allele-coupled exchange (ACE) vectors all conform to the modular format.
CHAIN Biotechnology has expertise working with anaerobic bacteria primarily for microbiome applications. We also supply a range of plasmid vectors and *Clostridium* strains, developed at the SBRC, to academic researchers and industry partners via our online store; www.chainbiotools.com. Furthermore, CHAIN offers support and bespoke services for plasmid transformation and cloning. CHAIN is uniquely positioned to support *Clostridium* focused research and help collaborators and customers accelerate development and commercialisation of new *Clostridium*-based technologies and products.

Conflict of interest: No

P 99

Global discovery of novel RNA-protein interactions in *Clostridioides difficile* using Grad-seq

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Clostridioides difficile (*C. difficile*) is an opportunistic enteropathogen that represents the leading cause of nosocomial antibiotic-associated diarrhea. The symptoms of an infection range from mild diarrhea to pseudomembranous colitis and tissue necrosis. Despite the important role of *C. difficile* in health care, the knowledge of mechanisms controlling its virulence is still low.
The discovery of regulatory networks comprising small noncoding RNAs (sRNAs) and RNA-binding proteins (RBPs) has altered our understanding of gene regulation in bacteria. It is now well established that bacteria widely use these molecules to regulate translation and mRNA decay. In recent years, several major RBPs and novel RNA classes were discovered, which act on cellular transcripts at different post-transcriptional stages. Many of them are important factors in the regulation of various metabolic, physiological and virulence-associated processes.
Our knowledge about RBPs and regulatory sRNAs in *C. difficile* is still at an initial stage. Recent studies revealed the existence of a large number of sRNAs, implying the presence of sRNA-based regulatory mechanisms in *C. difficile* (1). Phenotypic analysis of an Hfq knockdown strain suggested a role for this global RBP in different infection-associated pathways, such as biofilm formation and motility (2). However, our understanding of the precise role of Hfq in *C. difficile* is still incomplete and the existence of other RBPs remains unexplored.
Here, we combine density-gradient centrifugation of native cell lysates with RNA-sequencing and liquid chromatography-tandem mass spectrometry of individual fractions (Grad-seq) to identify functional non-coding transcripts and their associated RBPs in *C. difficile* (3). Comparative analysis of sedimentation profiles, such as clustering and dimension reduction, allows identification of functionally related transcripts. Based on these data we are able to predict novel RNA classes, RNA-protein complexes and global RBPs. We obtained sedimentation profiles of 3,452 transcripts and 1,879 proteins. The in-gradient profiles of housekeeping

RNAs, such as 5S, 6S and 16S, matched the expected sedimentation behavior. Most importantly, these RNAs co-sedimented with their associated RBPs.
Predicted sRNAs show a heterogeneous behavior and separate into several clusters of similarity. To identify possible binding partners of these similarly behaving sRNAs we established an RNA-bait-dependent pull-down approach. Additionally, we computationally identified proteins harboring RNA-binding domains that co-sediment along with clusters of similarly behaving RNAs. From this pool of predicted RBPs, we chose interesting candidates for co-immunoprecipitation. With this approach, we aim to discover novel RBPs, which we will further characterize concerning their role in metabolism, physiology and virulence.

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Conflict of interest: No

P 100

Developing novel therapies to prevent *Clostridium difficile* spore germination

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Clostridium difficile, recently renamed as *Clostridioides difficile*, is a Gram-positive obligate anaerobic bacterium. Ingested spores germinate in the gastrointestinal tract and the resulting vegetative cells secrete toxins that cause pseudomembranous colitis. Further shedding of spores results in transmission of this pathogen via the faecal-oral route. *C. difficile* infection (CDI) has become an urgent public health threat due to the rise in antibiotic resistant strains and the emergence of hypervirulent strains. To develop new therapies that target the first stage of pathogenesis, spore germination, it is imperative that the genetic mechanisms of *C. difficile* sporulation are identified. In order to recognize sporulation and germination related genes we have conducted random mutagenesis in several *C. difficile* strains. For that purpose we utilize a variety of plasmids and novel promoter systems to optimise efficiency. High-throughput screening of the transposon library for mutants defective in germination followed by high-throughput mapping enables the simultaneously identification of disrupted genes responsible.
Further characterisation of these genes will be performed by generating in-frame deletions by CRISPR-Cas9, complementing the mutants and further examining their deleterious affects on germination.

Conflict of interest: No

P 101

Clostridioides difficile toxin-mediated remodeling of the intestinal barrier increases adherence of *C. difficile* spores to the intestinal mucosa

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C. difficile is the primary cause of antibiotic-associated diarrhea worldwide and exhibits high rates of recurrence of the infection. *C. difficile* spores are considered as the morphotype of initiation, transmission, and persistence of the disease during recurrence of the infection. However, the mechanism of how *C. difficile* spores persist in the host remains unclear. During infection, *C. difficile* produces toxins TcdA and TcdB, which cause loss of cellular polarity and disruption of tight junctions leading to exposure of extracellular matrix proteins. Previously, we have demonstrated that *C. difficile* spores interact with fibronectin (FN) and vitronectin (VN) and that this interaction increases the spore adherence to intestinal epithelial cells. Currently, it is unclear if *C. difficile* toxins, through remodeling of the intestinal epithelial, may contribute to adherence of *C. difficile* spores to the intestinal mucosa. In this context, this work explores how toxins TcdA and TcdB contribute to the adherence of *C. difficile* spores in the intestinal mucosa.

By fluorescence confocal microscopy, we demonstrate that intoxicating monolayers of intestinal epithelial Caco-2 cells, increased cellular-accessible, but not total protein, of FN and VN. We also evidenced that intoxicated cells secrete FN and VN that is able to interact with *C. difficile* spores. Furthermore, in an ileal loop mice model, we observed in non-intoxicated ileal loops the presence of exposure sites of FN and VN. In TcdB-intoxicated ileal loops, while significant decrease and increase in the total levels of FN and VN were observed respectively, no changes in the levels of accessible FN and VN were detected. In vitro experiments also demonstrated that intoxicated intestinal epithelial cells exhibited higher levels of adhered *C. difficile* spores. Our results demonstrate that *C. difficile* toxins contribute to the remodeling of intestinal epithelial cells and increase the adherence of *C. difficile* spores to the intestinal barrier.

FONDECYT 1191601; Doctoral Fellowship 21161395.

Conflict of interest: No

P 102

Roles of the domains of *Clostridioides difficile* BclA proteins in exosporium assembly and spore-host interactions

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Clostridioides difficile spores are the morphotype of transmission, infection and persistence of *C. difficile* infections (CDI). The outermost spore-layer, called the exosporium, is involved with early interactions with intestinal epithelial cells, apparently through the hair-like extensions where the major structural components are the collagen-like proteins (BclAs). The laboratory *C. difficile* 630 strain harbors three orthologous collagen-like exosporium proteins (i.e., BclA1, BclA2 and BclA3); by contrast, most hypervirulent strains only encode BclA2 and BclA3 orthologues. These proteins have three distinctive domains: i) a N-terminal domain that anchors the BclA proteins to the exosporium surface; ii) a central collagen-like region (CLR) formed by GXX repeats; and iii) a C-terminal domain (CTD) of unknown function. However, the role of each domain in exosporium-assembly and interaction of the spore with components of the intestinal mucosa remains unclear. In this work, through allelic exchange, we constructed *C. difficile* R20291 isogenic mutant strains that lacked individual domains (CLR and CTD) in BclA2 and BclA3. Through transmission electron micrographs, we addressed how deletion of each individual domain affected exosporium assembly and the formation of the hair-like extensions. The role of each domain in the interaction of *C. difficile* spores with the extracellular matrix proteins, fibronectin and vitronectin, was assessed by immunofluorescence microscopy and solid phase binding assay. Significant differences in spore-adherence to intestinal epithelial Caco-2 cells were also observed upon deletion of the different domains of *bclA* ORFs. Notably, mutant strains exhibited lower resistance to heat and ethanol than wild-type spores, suggesting that the BclA exosporium proteins also contribute to *C. difficile* spore-resistant properties. All together, these results expand our knowledge of the role of each individual domain of the collagen-like BclA exosporium proteins.

Conflict of interest: No

P 103

Know your enemy: how anaerobic *Clostridium difficile* deals with low O2 tensions

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Clostridium difficile is a major cause of diarrhea associated with antibiotherapy. After germination of the spores in the small intestine, vegetative cells are exposed to several stresses including bile salts, antimicrobial peptides as well as reactive oxygen and nitrogen species produced by the host during inflammation. Apart from these different stresses, *C. difficile* also copes with oxygen (O_2) during gut colonization. Indeed, although the intestinal tract is regarded as mainly anoxic, low O_2 tension is present in the small and large intestines and tends to increase following antibiotic treatments. However, while strictly anaerobic, *C. difficile* is able to grow in non-strict anaerobic conditions (1-3% O_2) and tolerate brief air exposure indicating that this bacterium harbors an arsenal of proteins involved in O_2 detoxification and protection. We showed that *C. difficile* low O_2 tolerance requires the presence of the alternative sigma factor σB involved in the general stress response1. Among the different σB target genes, we focused on those encoding proteins involved in O_2 tolerance such as two reverse rubrerythrins (revrbr) and two flavodiiron proteins (Fdp). Among the four aforementioned proteins, only the Fdp, CD1623, has been functionally characterised2. We have purified the two reverse rubrerythrin and the second Fdp after overproduction in *Escherichia coli.* As previously observed for CD1623, both revRbr1 and revRbr2 harbours NADH: O_2- and H_2O_2-reductase activities in vitro while the second Fdp mainly acts as NADH: O_2-oxidase. Accordingly, inactivation of these different genes, either alone or in combination, results in *C. difficile* growth defect when exposed to low O_2 tensions. These findings demonstrate a key role for these four proteins in the ability of *C. difficile* to grow in presence physiological O_2 tensions. Work is in progress to test the ability of mutants inactivated in these oxygen reductases to colonize the mice.

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Conflict of interest: No

P 104

Proteolytic activity of *Clostridium difficile* HtrA regulates *htrA* expression levels, but not sporulation

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The *C. 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 *C. difficile* HtrA knockout is more virulent in hamsters, because of an increase of toxin levels. The knockout also displayed lower sporulation and diminished binding to target cells.
Using standard biochemical methods and a novel Nano-luciferase based approach, we show the localization of HtrA to be membrane bound, yet extracytoplasmic.
To dissect the relative importance of HtrA's protease vs. foldase activity for one or more of the abovementioned phenotypes, we have made a *C. difficile* mutant strain in which only the proteolytic activity of HtrA was abolished (S217A).
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.
We have developed a colony W-blot method to measure expression levels of HtrA. Using this technique and random transposon mutagenesis, we plan to investigate which proteins are involved in regulation of HtrA levels.

Conflict of interest: No

P 105

Clostridium botulinum risk in processed chilled foods

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The most feared food pathogen is *Clostridium botulinum* that produces the botulinum neurotoxin (BoNT). Even in nanogram concentrations BoNTs block neurotransmission and cause potentially lethal flaccid paralysis. *C. botulinum* spores prevail in environment and may occasionally contaminate food raw materials. The spores survive most food processing steps and may germinate into active toxigenic cultures during food storage. Some strains of *C. botulinum*, namely Group II strains, can grow and produce BoNT at chilled temperatures.
Control of the safety risk caused by Group II *C. botulinum* in chilled foods relies mainly upon heat processing and refrigeration. According to European recommendations, heat treatments applied for foods with shelf lives exceeding 10 days should kill 10^6 *C. botulinum* Group II spores. However, the natural spore contamination levels are several log-units lower than 10^6, and epidemiological evidence suggests a high safety record for most chilled foods. In line with sustainable goals and consumer preferences towards less processed food, the food industry seeks to optimize their heat treatments and to find alternative ways to control product safety. In an attempt to reassess the *C. botulinum* risk related to production of packaged chilled foods, we conducted a series of experiments representative of the key steps in food production: (i) prevalence of *C. botulinum* spores in 700 samples of food raw materials, (ii) heat-resistance of *C. botulinum* Group II spores at pasteurization temperatures, and (iii) growth and toxin production from heated and non-heated Group II spores at refrigerated temperature. The data suggest very small prevalence and low contamination levels of *C. botulinum* spores in Finnish food raw materials. Spore heat resistance studies revealed heterogeneity in the apparently isogenic spore populations, showing spore subpopulations with distinct heat destruction kinetics. Ongoing growth studies at refrigeration temperature suggest strain differences in growth lag, and interdependencies between efficacy

of heat treatment or initial spore count and time to growth. The results provide an experimental basis for a novel *C. botulinum* risk assessment model and help to optimize industrial processes applied in production of chilled foods.

Conflict of interest: No

P 106

Wide cellular heterogeneity in neurotoxin production and sporulation in *Clostridium botulinum* cultures

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Botulinum neurotoxin (BoNT) production and sporulation are the defining traits that make *Clostridium botulinum* a serious health threat and a concern for food safety. The two traits are connected at least via Spo0A, the master regulator of sporulation, which we recently showed to regulate also BoNT production(1). While it is generally understood that both BoNT production and sporulation are initiated in *C. botulinum* cultures at the end of logarithmic growth phase, it is unclear how the two traits are coordinated in and between cells and how environmental cues affect this coordination. We used single-cell gene expression analysis of *C. botulinum* wild-type and *spo0A* mutant strains in fluorescence microscopy to detect BoNT gene promoter activation in sporulating and non-sporulating cells and cultures exposed to various environmental conditions. BoNT and spore production were monitored along with microscopy. In general, we detected major cellular heterogeneity in BoNT expression and sporulation in the cultures. The dynamics of this heterogeneity was significantly affected by growth phase, medium composition, temperature, and the presence of growth-inhibitory factors. BoNT production and sporulation appear to be independently activated cellular programs. While both programs are under Spo0A regulation, a small cell subpopulation appears to regulate BoNT production independent of Spo0A. The results provide novel insights into clostridial neurotoxin production and sporulation and their regulation.

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Conflict of interest: No

P 107

Development of a nucleic acid-based assay for detection of *Clostridium botulinum*

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Clostridium botulinum causes a flaccid paralysis known as botulism via the production of botulinum neurotoxins (BoNTs). While *C. botulinum* produces several types of BoNTs (A-G), the majority of human botulism associates with BoNT types A, B, E, and F. Here, we developed a nucleic acid-based assay for detection of *C. botulinum* through multiplex polymerase chain reaction (mPCR) and multiplex real-time PCR. Multiplex primers and molecular beacon probes have been designed for BoNTs A, B, E and F genes. Then, the specificity of designed primers has been examined with other food pathogens. Next, annealing temperature and reagent concentration have been optimized for the optimal mPCR reaction. From our results, nucleic acid-based detection was more specific and less time-consuming than culturing method. Furthermore, we will compare the multiplex assay with gold-standard mouse bioassay. This multiplex assay is fast and cheap and requires less labor work, which could be useful to detect this pathogen in outbreaks.

Conflict of interest: No

P 108

Utilization of Cas9 nickase (Cas9n) to investigate the Clp proteases of *Clostridioides difficile*

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Clostridioides difficile is a Gram-positive, spore forming bacterium that has become the leading cause of hospital-acquired, antibiotic-induced diarrhea. Over the past several years CRISPR-Cas9 systems have revolutionized genetic editing abilities in a diverse set of organisms and have recently been utilized in *C. difficile*. Although CRISPR-Cas9 systems are relatively simple and can provide diverse user-defined alterations, the mechanism by which Cas9 cleaves DNA results in double strand breaks (DSB) that are known to be lethal in bacteria, which remains an unaddressed problem in bacterial mutagenesis. Therefore, we have explored the potential of a Cas9-nickase (Cas9n) based system that utilizes a Cas9D10A mutant to cleave only one DNA strand during the gene editing process. As a proof of concept, we have successfully used the Cas9n system to alter or fully delete five genes in *C. difficile* 630 and have characterized phenotypic changes in each of the mutants. In particular, we have used the Cas9n to generate single and double mutants in the *clpP1* and *clpP2* genes which encode for the two ClpP proteases in *C. difficile*. Further interrogation of the mutants revealed abnormalities in spore formation and reduced survival during heat stress. The Cas9n system provides a simple and versatile genetic manipulation strategy that can be used to improve our understanding of the pathogenesis of *Clostridioides difficile*.

Conflict of interest: No

P 110

Control of the mother cell line of gene expression in *Clostridioides difficile* by a conserved sporulation-specific cysteine protease

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yabG is the only sporulation-specific gene under the control of the late mother cell regulatory protein σK that contributes to a genomic signature for endosporulation. *yabG* codes for a cysteine (thiol) protease previously shown to be involved in the processing of a group of *Bacillus subtilis* spore coat proteins. In *Clostridiodes difficile*, YabG directly processes proteins involved in the germination response of spores to bile salts. Moreover, it is also required for the expression of genes involved in the late stages of coat/exosporium formation, including *cotA* and *cdeM,* and as such, spores formed by yabG mutants are structurally deficient. A model is proposed in which YabG proteolytically removes a regulatory protein that acts as a repressor of the expression of *cotA* and *cdeM*. This repressor appears to be SpoIIID which binds to the *cotA* and *cdeM* promoters in vitro and is cleaved by YabG. Thus, YabG appears to be a key factor in the regulation of the mother cell line in gene expression in *C. difficile*. It has been shown that in *B. subtilis,* σK sets up a negative regulatory loop that leads to a decrease in the levels of SpoIIID and proper coat gene expression. We show that YabG also cleaves the *B. subtilis* SpoIIID protein. This suggests that the σK-dependent transcription of *yabG* also leads to SpoIIID depletion in *B. subtilis* and thus, that the role of YabG in spore assembly is largely conserved. Since YabG is a sporulation-specific protease, it may represent a target to interfere with proper spore formation by pathogenic spore-forming organisms such as *C. difficile*.

Conflict of interest: No

P 111

Mechanisms of Carbapenem resistance in *Clostridioides difficile*

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Clostridioides difficile has an unusual peptidoglycan (PG), in that over 70% of the cross-links occur between the residues present in the third position of the stem peptides (3'-3'-type). These cross-links are formed through the action of L,D-transpeptidases (L,D-TPases). In contrast, the canonical D,D-transpeptidases (D,D-TPases) catalyse 3'-4'-type cross-links found in most bacteria. L,D-TPases are inhibited by carbapenems, a class of β-lactams, while D,D-TPases (penicillin-binding proteins) are inhibited by all classes of β-lactams. Carbapenems such as imipenem (IMP), are currently widely used in hospital settings to fight infections caused by Gram-negative bacteria. The emergence of carbapenem-resistant *C. difficile* strains thus poses a serious threat, since antibiotic resistance plays a major role in the infection and dissemination of this pathogen. We characterized two strains of *C. difficile* from ribotype R017 that show high level resistance to IMP.
We show that in these strains: i) the D,D-TPases PBP1 and PBP 3 show amino acid substitutions thought to confer resistance to the antibiotic; ii) β-lactams of different classes induce synthesis of a class D β-lactamase; iii) β-lactams also induce production of an extra PBP (found only in R017 strains), termed PBP 5, which, as we show, does not bind a fluorescent carbapenem. We further show that fluorescent vancomycin (Van-FL) which stains D-Ala-D-Ala motifs in PG, labels mainly the division septum, suggesting that the D,D-TPases are essential for the synthesis of PG during cell division and that the L,D-TPases synthesise the PG along most of the cell. In the presence of IMP, a susceptible strain forms shorter and curved cells consistent with impaired cell elongation. In resistant strains growing in the presence of IMP, however, Van-FL now decorates the entire cell contour. This suggests that the altered D,D-TPases now ensure PG synthesis along the entire cell surface and consistent with this idea, D-Ala-D-Ala muropeptides now accumulate. Carbapenem resistance in *C. difficile* thus appears to involve at

least three mechanisms: mutations in the genes coding for D,D-TPases, and the antibiotic-induced production of an extra PBP and a β-lactamase.

Conflict of interest: No

P 112

C. difficile carriage in dogs: a One Health issue?

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Clostridium difficile infection (CDI) is most frequently associated with infection within the healthcare setting and is responsible for most cases of antibiotic associated diarrhoea worldwide (1). Within veterinary species the extent of colonisation and disease varies considerably, with disease most prevalent in horses and young pigs (2). Both toxigenic and atoxigenic strains of *C. difficile* have also been isolated from dogs with and without diarrhoea (3). Consequentially, the clinical significance of isolating *C. difficile* in a stool sample from a diarrhoeic canine patient is currently unclear.
The primary aim of this study was to investigate the impact of *C. difficile* colonisation on canine gastrointestinal health based on isolation and toxin detection in stool samples from diarrhoeic and healthy individuals. A secondary aim is to clarify the role of *C. difficile* carriage by dogs to public health through comparative whole genomic sequence analysis.
Our results, although preliminary, confirm the previously reported prevalence of *C. difficile* carriage in dogs. We also found that colonisation by toxigenic strains actively producing toxins does not always correlate with diarrhoea in this species. Finally, the genomic relatedness of canine and human isolates hints at a possible interspecies transmission.
Our results, together with the high frequency of community acquired CDI of unknown origin, support the hypothesis that dogs may act as asymptomatic sources of infection.

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Conflict of interest: No

P 113

Microbial isolation from freshly-donated versus frozen fecal material of the same donor

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Several clinical and non-clinical technical studies have begun to test whether fresh fecal material is necessary or more efficacious for clinical resolution of diseases such as recurrent *Clostridium difficile* infection, inflammatory bowel disease, metabolic disorders and mental health conditions (1-3). As oxygen stress and freeze-thaw cycles are known to affect the survival of microbes, Vedanta Biosciences wanted to examine if the cultivability of microbes from a donor stool was significantly affected by Fecal Microbiota Transfer (FMT) processing and a single freeze-thaw of feces suspension stored in 10% glycerol.
Microbiologists from Vedanta Biosciences performed matching isolations from the same donor stool at the donation site (Leiden University Medical Center, Netherlands) and Vedanta Biosciences (Cambridge, United States) with informed consent of the donor. Stool was collected on site, processed and then frozen within thirty minutes; an aliquot was placed into an anerobic chamber prior to processing and freezing for the 'fresh' isolation. At both locations donor stool was serial diluted and inoculated onto eight different selective and non-selective media, and incubated anaerobically at 37°C for 48hrs. A total of 1288 colonies were isolated by hand from the agar plates into Peptone Yeast Glucose (PYG) broth and incubated anaerobically for 48hrs. Following growth in PYG broth, isolates were subjected to broth-based colony PCR ('hot-shot' PCR) and then subsequent Sanger sequencing. Sanger sequencing data was then processed for species-level taxonomic assignment.
A general 10-fold loss in cultivability of anaerobic bacteria was found during the processed/frozen isolation; all media types displayed optimal dilution plates at one dilution less than the fresh isolation. Thirty-six species were confidently (multiple high-quality isolates/sequences of each species) identified in both the fresh and processed/frozen isolation campaigns. An additional 26 species were confidently identified exclusively in one of the two isolation campaigns. Notably of the 26, *Bacillus* spp. and *Anaerostipes hadrus* were identified numerous times in the 'fresh' isolation but not in the processed/frozen isolation, while for *Hungatella*

effluvii the reverse was true. We conclude that a 10-fold loss of overall FMT viability was found due to the processing and freeze thaw, indicating that under certain clinical indications other than rCDI fresh, specifically-processed, or a defined microbial cocktail may be required for efficacy.

1 JAMA. 2016;315(2):142-149
2 Infect Dis Ther (2018) 7:71–86
3 EBioMedicine 41 (2019) 509–516

Conflict of interest: Yes

Disclosures: Employee of Vedanta Biosciences

P 114

Effect of fecal microbiota transplants to transfer and eradication of antibiotic resistance genes in recipients

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Fecal Microbiota Transplant (FMT) has been the subject of extensive research in the recent years. It has successfully been applied as treatment in patients with multiple recurrent *Clostridium difficile* infection (CDI). The general accepted mechanism behind this treatment is the repression of *C. difficile* through the newly implanted microbiota, which leads to a general stabilization of the microbiota in the patient. An additional effect also has recently received more attention, namely eradication of antibiotic resistant bacteria. CDI patients often harbor an increased amount of proteobacteria which can harbor various antibiotic resistance (ABR) genes. Therefore the question arises, if a fecal microbiota transplant makes it possible to eradicate these antibiotic resistant organisms. On the other hand, it is also possible that antibiotic resistant organisms are transferred from the donors to the patients.

FMT was performed in 55 patients with multiple rCDI using a cohort of 9 well screened healthy donors. Shotgun metagenomics was performed on an Illumina Nextseq on donor fecal samples, and on patient fecal samples before and after FMT. The sequencing data was quality controlled and taxonomically profiled with the One Codex platform. Afterwards all reads were cross-assembled with the MegaHit assembler, annotated for ABR genes with Abricate, and taxonomically profiled with blastn and a custom LCA algorithm.

In total 1811 ABR genes or virulence factors were identified. Most of these genes belonged to the order Enterobacterales (877), but also many to enterococci (97). Many genes (437) could not uniquely be assigned to at least a phylum. Tetracycline resistance encoding genes (*tetW, tetO, tetQ* and *tet40*) were present in (nearly) all samples (65-89%), including most donor samples, and belonged to the most abundant resistance genes, followed by aminoglycoside resistance, (AGly)Aph3-III, and multidrug efflux pumps (*acr* and *mdt*). All groups (donors, patients before and after FMT) harbored considerable amounts of ABR genes, with in general less

genes present in donors. While FMT decreased this amount by roughly 2/3 of the original concentration, it did not reach the amount of ABR genes present in donors. In some cases this decrease resulted in a decrease of abundance, while simultanesouly an increase of individuals containing these genes was observed. Proteobacteria were in general also still detected in most patients after FMT, but *Klebsiella* or *Morganella morganii* related ABR genes were not detectable in most patients. Most genes did not seem to be donor specific, and appeared in multiple patients before and after FMT. Two of the 9 donors contained 4 genes of the vanG operon. One of these donors was consistently colonized over 13 donations with the unidentified organism (*Streptococcus* or *Enterococcus*). In 3 cases the donors transferred the organism to a recipient.
While the recipient microbiota, including ABR genes, changed through FMT, it did not lead to a widespread eradication of these, but rather to a general reduction. Similarly, Enterobacterales decreased in the patients after FMT. Donors did not seem to contribute considerably to ABR gene spread.

Conflict of interest: No

P 115

Epsilon toxin from *Clostridium perfringens* induces intracellular calcium mobilization dependent on Myelin and Lymphocyte protein expression

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Epsilon toxin (Etx) from *Clostridium perfringens* toxinotypes B and D is the third most potent toxin after botulinum and tetanus toxins, it has an economic impact on livestock and is considered a potential bioterrorism agent.
Etx produces acute perivascular edema affecting several organs, especially brain and lungs. At a cellular level, Etx has a cytotoxic effect on defined cell models, being the Madin Darby Canine Kidney cell line the most extensive *in vitro* model used due to its extreme sensitivity to the toxin.
Etx belongs to the family of pore-forming bacterial toxins that requires a specific receptor on the cell surface to exert its cytotoxic effect. The oligomerization of the toxin on the plasma membrane would alter the cell's integrity and cause an ionic imbalance. Among the potential receptors for Etx, the Myelin and Lymphocyte protein (MAL) arises as the most promising candidate. Accordingly, cell lines expressing MAL protein were selected and incubated with Etx and this effect was compared with cell lines not expressing MAL protein. In addition, we have explored the ability of human MAL to sensitize originally non-responding cell lines to Etx by inducing its expression or to desensitize naturally expressing MAL cell lines by preventing its expression. The cytotoxic effect of Etx was studied by a cytotoxic assay and by monitoring the toxin dependent ATP release.
The toxin produced a cytotoxic effect and induced the release of ATP only in MAL expressing cell lines and this effect of Etx was linked to the formation of Etx oligomer. Furthermore, the intracellular cascades triggered by the interaction between Etx and MAL protein were studied by expressing MAL in oocytes and monitoring the possible ionic currents and ATP release induced by Etx. We observed that ATP release induced by Etx was dependent on the intracellular Ca2+

mobilization. Moreover, this Ca2+ mobilization activated the endogenous calcium dependent chloride channels of *Xenopus* oocytes which are also described as TMEM16A or Anoctamin-1. However, despite intracellular Ca2+ mobilization, no ionic currents associated with Etx pore formation were recorded at a membrane potential of -40 mV.
All together, these results confirm the requirement of MAL protein expression for the cytotoxic effect of Etx. Furthermore, additional mechanism dependent on Etx-MAL interaction, besides pore formation, may exist promoting intracellular Ca2+ mobilization and ATP release.

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Conflict of interest: No

P 116

Construction and validation of naturally atoxigenic *Clostridium botulinum* strains for use in food challenge studies

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Clostridium botulinum is a foodborne pathogen that is notorious for its ability to produce a deadly neurotoxin and cause botulism when it grows in foods. Although the incidence of foodborne botulism is low, the pathogen is important because it serves as a reference organism for designing pasteurisation and appertisation treatments. When food producers design novel foods with altered composition or new ingredients or develop new processing and preservation techniques, challenge studies with *C. botulinum* may be required to validate their safety. Depending on the food and the way it is processed and stored, these tests will be conducted with group I *C. botulinum,* which forms very heat resistant spores, and/or with group II *C. botulinum,* which is a psychrotroph that can grow in refrigerated foods. However, conducting challenge studies at pilot production scale is cumbersome and costly because of the possible formation of highly toxic products. To make challenge studies more accessible, this project aims at developing strain cocktails of naturally atoxigenic surrogate strains both for group I and group II. Such strains have been reported but they have been poorly characterized. Therefore, 77 environmental and food samples were processed with an in-house optimized protocol to isolate *C. botulinum* strains. So far, 20 group I and 10 group II strains have been obtained and identified by 16S rDNA sequencing. These strains, and an additional 12 atoxigenic group II strains isolated previously at the Robert Koch Institute were further analysed by whole genome sequencing. This confirmed the grouping for 33 strains (other strains in progress), and also indicated the absence of a botulinum neurotoxin gene cluster in these strains. Multilocus sequence typing analysis showed that the group II atoxigenic isolates represented the full phylogenetic breadth of WGS available in Genbank, while the isolates of group I were most closely related to *C. sporogenes*, which forms a specific subcluster within group I *C. botulinum.* Furthermore, a phenotypic characterization was initiated in which the formation, germination and heat resistance of the spores, and the minimal temperature, water activity and pH for growth, are being analysed. This analysis confirms so far that the properties of the strains align with their genetic grouping. Further comparison of genotypic and phenotypic information will be used to select representative strains for challenge studies. This research addresses a need that will stimulate innovation in the food industry, and may also provide new insights in the ecology and evolution of *C. botulinum* and botulinum neurotoxin genes.

Conflict of interest: No

P 117

Characterization of the serine/threonine kinase PrkC of *Clostridium difficile* and identification of kinase substrates

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Clostridium difficile is the leading cause of intestinal nosocomial post-antibiotic infections in adults. During infection, the bacterium must rapidly respond and adapt to the host environment by using survival strategies. Protein phosphorylation is a reversible post-translational modification employed for signal transduction and regulation. Bacterial serine/threonine kinases (STKs) and their associated phosphatase (STP) play an essential role in the regulation of many different processes in bacteria, such as growth and cell division, cell wall biosynthesis, antibiotic tolerance, stress response, metabolic and developmental processes and virulence. We have previously shown that the STK PrkC is membrane associated and localizes at the septum. Deletion of PrkC affects cell morphology with an increased size and the presence of abnormal septa. Moreover, the properties of the cell envelope are affected in the *ΔprkC* mutant as well as the resistance to antimicrobial compounds targeting cell wall. This increased susceptibility is not associated with differences in the structure of the peptidoglycan or polysaccharide II. However, the *ΔprkC* mutant contains a reduced amount of peptidoglycan. Finally, the *ΔprkC* mutant has a delay in gut implantation in a hamster model (1). By comparing the phosphoproteome of the wild-type strain and the *ΔprkC* mutant, we identified three potential substrates of PrkC corresponding to proteins that participate in different cell processes affected in the *ΔprkC* mutant. This result suggests that PrkC in *C. difficile* has a pleiotropic effect by targeting multiple pathways. Among PrkC targets, IreB is a small protein of unknown function that is well conserved in Firmicutes. IreB has been identified as a negative regulator of cephalosporin resistance in *Enterococcus* faecalis (2). We demonstrated *in vivo* and *in vitro* that IreB was specifically phosphorylated by PrkC at Threonine

8. Interestingly, using size-exclusion chromatography we observed that phosphorylation of IreB promotes its oligomerization. To determine the specific role of IreB in *C. difficile*, a *ΔireB* mutant was constructed. However, this mutant exhibited similar level of cephalosporin resistance than the wild-type strain. Although we do not yet understand the function of IreB, we observed that growth is impaired by the introduction of an expression vector encoding IreB or IreB-T8A (phospho-ablative) into a *C. difficile* strain lacking the PrkC kinase. These data suggest that a high level of un-phosphorylated IreB is toxic for the cells and that phosphorylation by PrkC could be a mechanism of inactivation by changing the oligomerization state of IreB. The two other PrkC substrates, a protein of the divisome and an enzyme probably involved in peptidoglycan metabolism are also currently studied.

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Conflict of interest: No

P 118

Diarrheagenic activity of each component of CPILE as a new enterotoxin produced by *Clostridium perfringens* isolated from foodborne outbreaks

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We identified the novel diarrheagenic enterotoxin, named *Clostridium perfringens* iota-like enterotoxin (CPILE), as the causative agent of the foodborne outbreaks by *C. perfringens*. CPILE consists of two components, CPILE-a and CPILE-b. CPILE belongs to two component toxins, such as *C. perfringens* iota toxin that consists of I-a and I-b. In this study, diarrheagenic activity of each CPILE component was investigated.
Trypsin-treated CPILE-a, trypsin-treated CPILE-b and chymotrypsin-treated I-b were investigated. The diarrheagenic activity of the individual component of the toxins was determined by the use of a rabbit ileal loop test.
Fluid accumulation (FA) clearly occurred in the loop inoculated with trypsin-treated CPILE-b. The FA ratios (ml liquid/cm length of loop) of trypsin-treated CPILE-b (10μg, 50μg and 100μg) were 0.3 to 1.3, 1.3 to1,4 and 1.7, respectively. On the other hands, chymotrypsin-treated I-b (50μg and 100μg) did not evoke FA. Trypsin-treated CPILE-a (50μg) did not also evoke FA.
These findings indicated that CPILE-b itself possesses diarrheagenic activity and evokes FA without any co-operative function of CPILE-a. CPILE would not be the typical two component binary toxin such as iota toxin and iota-like toxin.

Conflict of interest: No

P 119

Public health risks related to type C botulism in dairy cattle: a case report

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In March 2018, a bovine botulism outbreak occurred in the province of Bolzano, North of Italy.
The herd was composed by 53 bovines divided into 3 boxes. The fist box hosted 27 lactating cows and one peri-parturient heifer. The second box was divided from the first box by means of the feeding alley and hosted 19 heifers and 2 dried cows. The third box with 4 calves was located in a different cowshed.
The diagnosis based on clinical remarks (anorexia, hypothermia, drop in milk production, sialorrhoea, legs weakness, initial mild diarrhea followed by constipation, difficulty in retracting the tongue, sternal/lateral recumbence, respiratory failures) and it was strengthened by the detection of non-chimeric type C botulinum neurotoxin (BoNT) encoding genes in the enrichment broths previously inoculated with liver and intestine samples collected from the first dead subject. Biomolecular investigations excluded the presence of BoNT type A, B, D, E and F encoding genes. Three days after the beginning of the symptoms, the spoils of a cat were found in the manger of the lactating cows. Both the carcass and the total mix ration collected from the manger resulted positive for non-chimeric type C BoNT encoding genes.
Five days after the onset of the disease, all the lactating cows showed symptoms referable to botulism and serum, milk and feces were collected from 12 subjects. Serum and milk samples were screened for BoNTs through the mouse test and EndoPep-MS. The same laboratory investigations were performed in 6 and 9 milk and serum samples collected respectively after 8 and 14 days from the index case. Real-time PCR protocols for BoNT encoding genes types A to F were applied to enrichment broths previously inoculated with the fecal samples and with the tank milk collected after 8 and 16 days from the onset of the outbreak.
All serum and milk samples resulted negative for BoNTs and the tank milk samples

were PCR-negative. On the contrary, 10 out 12 fecal samples tested positive for type C BoNT encoding genes.
The outbreak lasted 14 days and 21 cows (20 lactating cows and 1 heifer) died (almost the 40% of the herd).
Our results demonstrate that BoNTs and BoNT producing Clostridia are not present in the sera and notably in the milk collected during the outbreak described, nor in symptomatic animals neither in cohort animals that subsequently showed clinical signs or died.
In conclusion, the public health risk related to dairy products originated during a bovine botulism outbreak sustained by non-mosaic type C BoNTs appears very low.

Conflict of interest: No

P 120

Antimicrobial susceptibility of *Clostridium botulinum* group III field strains

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Although antibiotic treatments are discouraged in case of botulism, some antimicrobials are successfully used to treat poultry flocks affected by the toxico-infective form of the disease. In addition, information on the drug susceptibility of *C. botulinum* group III could be of help to improve selective media for the isolation of these fastidious anaerobes. The genetic characterization of strains isolated from biological and environmental samples in animal botulism outbreaks is of help for epidemiological investigations. In many European countries, where commercial vaccines to prevent animal botulism are not available, the isolation of the strain can result essential to produce auto-vaccines that are employed in prolonged or recurrent botulism outbreaks of farmed animals. In Italy, the authorization for the production of auto-vaccine is released by the Ministery of Health only to national public veterinary laboratories (Istituti Zooprofilattici Sperimentali).
In this study we report the drug susceptibility of 71 *C. botulinum* group III field strains collected within five different European countries. One strain was isolated from mink-feed (non-chimeric type D) and 70 strains were isolated from biological samples collected from animals affected by botulism or died showing neuro-paralytic symptoms compatible with botulism. Nineteen strains were isolated from bovines (7 type C, 12 type D/C); 14 from wild birds (13 type C/D and 1 type D/C); 35 from poultry (34 type C/D and 1 type D/C) and 2 from dogs (C/D). Three strains C/D and two D/C resulted not toxic at the time of testing but their identity as belonging to *C. botulinum* group III was confirmed by MALDI-TOF MS analysis. The minimum inhibitory concentration (MIC) was established through the agar dilution method according to the CLSI (Clinical and Laboratory Standard Institute)

manual M11-A8. Antimicrobials tested were penicillin, amoxicillin, tylosin, tiamulin, tetracycline, lincomycin, enrofloxacin, gentamycin, sulfamethoxazole with trimethoprim (SXT), vancomycin, polymyxin B, metronidazole. The reference strains *Bacteroides fragilis* ATCC 25285 and *Clostridium difficile* ATCC 700057 were used as quality control.
The lowest MIC values were recorded for penicillin (MIC_{50}≤0.016 mg/L; MIC_{90}=0.032 mg/L), amoxicillin (MIC_{50}=0.032 mg/L; MIC_{90}=0.125 mg/L); tetracycline (MIC_{50}=0.032 mg/L; MIC_{90}=0.063 mg/L) and lincomycin (MIC_{50}=MIC_{90}=0.063 mg/L). Gentamycin (MIC_{50}=32 mg/L; MIC_{90}=64 mg/L), SXT (MIC_{50} and MIC_{90}>256 mg/L) and polymyxin B (MIC_{50} and MIC_{90}>256 mg/L) showed the highest MIC values. MIC_{50} and MIC_{90} were 0.125 mg/L for tylosin and 0.25 mg/L for vancomycin. Enrofloxacin showed MIC_{50}=0.063 mg/L and MIC_{90}=0.125 mg/L, tiamulin MIC_{50}=0.125 mg/L and MIC_{90}=0.25 mg/L and metronidazole MIC_{50}=0.125 mg/L and MIC_{90}=0.5 mg/L. The MIC_{50} matched or differed not more than one or two dilutions from MIC90 of the same antimicrobial, due to a linear distribution of the MIC values of the strains, irrespective of the geographical origin, the animal source and the toxinotype of the strain. Our results confirm the good "in vitro" efficacy of β-lactams that represent the first choice of treatment in poultry flocks with botulism. We recorded high MIC values only for gentamycin, SXT and polymyxin that are usually active against Gram-negative bacteria. Gentamycin and vancomycin could be promising antimicrobials for the development of a selective medium for *C. botulinum* group III.

Conflict of interest: No

P 121

First cross-national outbreak of foodborne botulism observed in the EU due to a commercial product

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Between November and December 2016 a cluster of six cases from five households of foodborne botulism type E due to the consumption of salt-cured, dried roach (*Rutilus rutilus*) occurred in Germany and Spain and a potential seventh case was noticed in April 2017 in Germany. All persons were of Eastern European (Russian and Kazach) backgrounds and purchased the fish, which was consumed unheated as a snack, from an international wholesaler specialised on Eastern European products. Identification of *Clostridium botulinum* type E and/or BoNT/E from the consumed fish initiated an European-wide food recall through the EU Rapid Alert System for Food and Feed. Despite the timely recall two cases occurred afterwards indicating that consumers are not always aware of food reached notices in particular if they visit certain shops only infrequently.
Trace-back-analysis to identify the original producer was hampered by the fact that four different products of dried roach originating from various fishing grounds were sold as lose ware simultaneously. Three distinct producers from the Netherlands and Lithuania processing fish from the Netherland, Lithuania and Poland were identified.
Furthermore the BoNT subtype of the isolates from patients and fishes were obtained and compared. Interestingly, two different subtypes, E1 and E3, were identified. Both subtypes are the most common subtypes encountered at the Baltic and Northern Sea, therefore giving no further evidence with respect to the

origin of the contamination. Noteworthy, one patient harboured two different strains, a BoNT/E1 and an E3 strain indicating that food can be simultaneously contaminated with more than one strain of *C. botulinum*.
Fish like roach can be naturally contaminated by spores of *C. botulinum*, in particular in their intestines and as such uneviscerated fish may be of higher risk. Consumption of unheated dried fish such as roach is occasionally associated with botulism. However, salt-curing and drying does usually prevent germination, growth and toxin production of *C. botulinum*. On how the natural spore contamination developed to an unsafe product can only be speculated. Either reduction of the free water level by the salting and drying process was not effective throughout the fish, thus creating pockets of suitable growth conditions for *C. botulinum* or the germination growth and toxin-production occurred prior to the salting process, e.g. during storage under non-refrigerated-temperature conditions.

Conflict of interest: No

P 122

Screening of natural antimicrobial compounds against *Clostridium botulinum*

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Clostridium botulinum is an anaerobic, spore-forming, gram-positive bacterial pathogen known for its production of a potent neurotoxin that can be fatal to humans and animals at low concentrations. In order to prevent outbreaks of foodborne botulism, effective methods are required to eliminate *C. botulinum* by processing or to control its outgrowth by adequate food formulation or use of antimicrobials.

Here, we report the antimicrobial activity of eleven plant-derived Natural Antimicrobial Compounds (NACs) (alpha-terpineol, carvacrol, citral, eucalyptol, eugenol, geraniol, hop beta acids, linalool, linalool oxide, pinene and t-cinnamaldehyde) against *C. botulinum* Group I (GICb) and Group II (GIICb), the two phylogroups involved in human foodborne botulism.

Carvacrol, geraniol and eugenol had MIC values of 275 ppm, 500 ppm and 1200 ppm respectively against both botulinum groups and eight gram-positive bacteria included as controls (*Listeria monocytogenes*, two *Bacillus* species and five *Staphylococcus* species). Citral inhibited GICb at 251 ppm and GIICb at 502 ppm, and controls were inhibited at the same concentrations. Alpha-Terpineol was tested against GIICb only and had a MIC of 1052 ppm while it did not inhibit non-botulinum bacteria up to 4208 . Linalool inhibited all GICb strains at 2025 – 4050 ppm while it only inhibited only five out of twenty GIICb strains. Linalool oxide, pinene and eucalyptol did not show growth inhibition up to 4253 ppm, 3861 ppm and 4151 ppm respectively on both groups.

For three compounds (carvacrol, t-cinnamaldehyde and hop beta acids), the effect on spore germination was tested for two GIICb strains. The compounds had MIC values of 300 ppm, 500 ppm and 2.5 ppm respectively. Carvacrol and t-cinnamaldehyde showed inhibition of spore germination at much lower concentrations, 8 ppm and 30 ppm respectively. Hop beta acids, in contrast, did not show reduction of spore germination up to 100 ppm, which represents 40 times the determined MIC.

Overall, we conclude that GICb and GIICb have similar sensitivity to all the tested compounds, and that there are no important strain-to-strain differences. The MIC values are also in the same range as for other gram-positive bacteria. The hop beta acids are of potential interest because they exhibit a MIC value at least 100 times lower than the other tested compounds. Although not all compounds have been tested yet, there appears to be a remarkable difference in the effect of the compounds on spore germination. Further work will be needed to understand the mode of action of these natural antimicrobials against vegetative cells and spores.

Conflict of interest: No

P 123

Successful *Clostridioides difficile* ribotyping directly on faecal samples

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Clostridioides difficile is the most common cause of nosocomial diarrhoea. Hospital outbreaks occur regularly due to the high number of bacterial spores excreted by symptomatic patients. To detect transmission of strains between patients, bacterial isolates are typed. PCR ribotyping is the most widely used technique for typing of *C. difficile*. With this method, strains are distinguished based on number and lengths of the ribosomal 16S-23S intergenic spacer regions. Ribotyping is usually performed on cultured strains of *C. difficile*. The objective was to develop a ribotyping method that could be applied directly on faecal DNA and would eliminate the need for culture. Optimally, the technique should be as sensitive as qPCR to use it as first-line diagnostic assay. Our secondary objective was to combine direct ribotyping with *C. difficile* toxin genes detection.
We designed a highly specific and sensitive primer set to perform ribotyping directly on total faecal DNA. Toxin primers were adapted from published work by Persson et al [1]. DNA isolation was performed with MagNaPure96. PCR ribotyping and toxin detection was applied to 65 faecal samples, that were proven positive for *C. difficile* toxins by a toxin-specific qPCR, and to their 65 corresponding cultured strains. Sixty-seven control samples were used; these were all negative for *C. difficile* but 14 were positive for other enteropathogens, such as *Campylobacter*, *Salmonella* or norovirus. PCR products were analysed by capillary gel electrophoresis on an ABI 3500 machine. DNA fragment peak profiles were visualised with TIBCO Spotfire software. Clustering was performed by Unweighted Pair Group Method with Arithmetic Mean with cosine correlation as distance measure and average value as ordering weight.
Ribotype peak profiles were observed in all 65 *C. difficile* positive faecal samples and strains, indicating high sensitivity (n=65/65). No peaks were observed in *C. difficile* negative samples, resulting in a specificity of 100% (n=65/65). Profiles of

faecal samples and strains were highly similar. Samples with identical ribotypes could be distinguished from samples with different ribotypes by cluster analysis. Toxin genes were detected in all *C. difficile* positive and in none of the *C. difficile* negative samples and presence of certain toxins in the different ribotypes corresponded to literature data.

C. difficile ribotyping directly on faecal DNA is feasible with sensitivity comparable to that of *C. difficile* toxin qPCR. This allows rapid diagnosis and rapid comparison of strains for detection of bacterial transmission between patients, without the need for culture. This may result in more timely application of infection control measures to limit *C. difficile* outbreaks.

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Conflict of interest: No

P 124

Genome sequencing and phylogenetic analysis of *C. sporogenes* strains

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Clostridium sporogenes is a gram-positive anaerobic bacterium that has high genomic identity with group I *Clostridium botulinum*. The key difference between the two organisms is the production of the potent botulinum neurotoxin (BoNT). BoNTs are the causative agent of botulism, a potentially deadly paralytic disease. The absence of the BoNT gene cluster in *C. sporogenes* makes it a valuable surrogate for Group I *C. botulinum*. To understand the diversity of C. sporogenes within our strain collection, we have sequenced four legacy strains of C. sporogenes, and determined their phylogenetic placement via multilocus sequence typing (MLST) and core genome MLST (cgMLST). Our analysis suggests strain CFSAN084860 represents a novel sequence type. CFSAN084861 represents a known sequence type and to the best of our knowledge, this is the first time a member of that ST has been fully sequenced. Finally, our analysis indicates that CFSAN084862, a PA3679 strain, does not significantly differ from the from the majority of other sequenced PA3679 isolates from most other labs and industry.

Conflict of interest: No

P 125

Diversity, origin, and microevolution of *Clostridioides difficile* BI/NAP1/027/ST1 strains from Latin America

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Clostridioides difficile BI/NAP1/027/ST1 has been responsible for outbreaks of antibiotic-associated diarrhoea in clinical settings worldwide and is associated with severe disease presentations and mortality rates. Previous work distinguished two lineages of fluoroquinolone-resistant epidemic BI/NAP1/027/ST1 strains that disseminated from North America to Europe and Asia (FQR1) and to Europe and Australia (FQR2) around ~1993 and ~1994, respectively. In this study, we generated 25 draft genome sequences for clinical BI/NAP1/027/ST1 isolates recovered between 2009 and 2016 in Costa Rica (n=16), Chile (n=5), Honduras (n=3) and Mexico (n=1) and compared them to 141 global isolates of FQR1 and FQR2 strains to elucidate their dispersion dynamics throughout Latin America (LA). We also sought to analyse whether the LA isolates exhibit local adaptations, with a particular emphasis on their mobile resistome due to its clinical relevance and potential role in adaptation and dissemination. A most recent common ancestor analysis revealed that the LA isolates analysed were most likely introduced independently from Europe (n=5) and to a lesser extent, from North America (n=1), between 2001 and 2006. Most of them belong to the FQR2 lineage (n=23, 92%). No transmission events between Latin American countries could be reconstructed from the dataset. Isolates from Chile, Honduras, and Mexico had

twice as many antibiotic resistance genes (ARG) as cognates from the rest of the world. Their unique set of ARGs includes *ermB*, *cfr* and *tet(W/N/W)*, which were found integrated into undescribed integrative and conjugative elements. These findings expand current knowledge on the genomic variation and microevolution of the BI/NAP1/027/ST1 strain and justify further surveillance of antimicrobial resistance in this pathogen in LA.

Conflict of interest: No

P 126

Fecal microbiota composition as predictor for recurrent *Clostridioides difficile* infection – a study protocol

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Clostridioides difficile infection (CDI) is the most common cause of hospital-acquired diarrhea. Despite adequate antibiotic treatment, approximately 25% of patients develop recurrent CDI (rCDI). This leads to severe morbidity, mortality, and high costs. The most important risk factor for developing rCDI is a disturbed gut microbiota. Fecal microbiota transplantation (FMT) is used to restore the microbiota composition and is a highly effective treatment for rCDI.
We aim to build a prediction model for rCDI based on gut microbiota composition in combination with clinical characteristics. This prediction model will help clinicians to stratify patients based on recurrence risk, to identify patients that could benefit from treatment with FMT for their primary CDI.
In a pilot study we were already able to identify changes at the bacterium phylum level related to disease status (recurrence (n=7) vs non-recurrence (n=14)). In patients who developed rCDI we observed a decrease in diversity of Firmicutes and Proteobacteria, with a concomitant increase in Bacteriodetes. This suggests that the microbiota composition on the day of diagnosis of primary CDI could help to stratify patients according to their recurrence risk.
We are performing a multicenter, prospective, observational study, including 150 patients with primary CDI. Fecal samples are collected on the day of diagnosis of the primary CDI and on day 4 to 6 after initiation of CDI treatment. In addition, data on patient characteristics, disease severity, *C. difficile* ribotype, CDI treatment, and medication use are collected. Patients are followed for eight weeks to evaluate rCDI occurrence. Microbiota analysis is performed by IS-pro™. With this PCR technique, bacterial species are distinguished based on number and lengths of the ribosomal 16S-23S intergenic spacer regions. The microbiota data, together with clinical parameters will be used to construct a prediction model for CDI recurrence risk. Subsequently, this model will be validated in a new group of 75

patients.

Conflict of interest: Yes

Disclosures: Andries Budding is an employee of InBiome B.V.

P 127

The smoking gun of *Clostridium difficile*

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Clostridium difficile is the major cause of antibiotic-associated diarrhoea worldwide. Disruption of the commensal microbiota, such as through use of broad-spectrum antibiotics, is key for colonisation by *C. difficile* and development of *C. difficile* disease (CDI). Furthermore, failure of the gut microbiota to recover diversity and re-establish a healthy microbiota, results in recurrence of infection. After an initial recurrent episode, patients are more likely to relapse multiple times. An unusual characteristic of *C. difficile* among gut bacteria is its ability to produce the bacteriostatic compound para-cresol through fermentation of tyrosine, via an intermediate para-hydroxyphenyl acetate (*p*-HPA). Here, we investigate the interaction of *C. difficile* with other commensal microbiota and the effect that *p*-cresol production by *C. difficile* has on their growth and microbial diversity. Using a gene inactivation mutant (*hpdC*::CT) of the decarboxylase responsible for the conversion of *p*-HPA to *p*-cresol, we demonstrate that *p*-cresol production provides a competitive advantage for *C. difficile* over commensal gut bacteria; *E. coli*, *K. oxytoca* and *B. thetaiotaomicron*. Metabolic profiling highlights the importance of acetate, butyrate, *p*-cresol and *p*-HPA in driving separation of wild-type *C. difficile* from a p-cresol mutant. Removing the ability to convert *p*-HPA to *p*-cresol leads to a fitness defect in a mouse relapse model of *C. difficile* infection. These mice have a distinctly altered gut microbiota composition, alongside altered faecal metabolite profiles. We demonstrate that Gammaproteobacteria are susceptible to exogenous p-cresol in-vitro and that there is a clear divide between bacterial Phyla and their susceptibility to *p*-cresol. The Gram-negative species were relatively sensitive to *p*-cresol, whereas Gram-positive species were more tolerant. This study demonstrates that production of *p*-cresol by *C. difficile* has an effect on relapse of CDI in a mouse model of infection, as well as the viability of Gram-negative commensal intestinal bacteria and metabolite production,

suggesting that *p*-cresol production directly or indirectly contributes to *C. difficile* pathogenesis.

Conflict of interest: No

P 128

Improving vaccination schemes against *Clostridium perfringens* type C enteritis in pigs

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Clostridium perfringens type C induced necrotizing enteritis (NE) causes high mortality in newborn piglets and immunization programs using commercially available vaccines are readily used to prevent disease. Sows are vaccinated during every gestation period and antibodies taken up from the colostrum passively protect newborn piglets. Although *C. perfringens* type C secretes many different toxins, antibodies against beta-toxin are most likely essential for protective immunity, as the toxin is essential for pathogenesis. Because the pathogen can persist for several years on farms, continuous vaccination combined with good management procedures are essential to maintain a low pathogen burden and to protect pig herds from the re-occurrence of the disease. Generally, primiparous sows are immunized twice during their first gestation, in order to induce a significant increase of anti-beta-toxin antibodies in the colostrum. Subsequently, multiparous sows are supposed to be re-vaccinated prior to each farrowing. To monitor neutralizing anti-beta-toxin antibodies in pigs after vaccination, we developed a cell culture assay for the quantification of neutralizing anti-beta-toxin antibodies (1). This test system was then applied in two subsequent field trials using commercially available vaccines in Switzerland. Most vaccinated sows developed neutralizing antibody titers against beta-toxin in serum and colostrum. However, a proportion of primiparous sows, when immunized according to the manufacturer's recommendations, which includes two injections of the vaccine during the first pregnancy, developed no or only very low neutralizing antibody titers. In contrast, all multiparous sows developed serum and colostral antibody titers after a booster vaccination shortly before their next farrowing. These were significantly higher when compared to primiparous sows. The colostral antibody titer of sows highly correlated with the serum antibody titer of their piglets after consumption of colostrum. An adapted vaccination scheme using 3 instead of 2 initial immunizations before the first farrowing of primiparous sows significantly improved antibody response in these animals. In conclusion, we demonstrate that despite following the generally accepted vaccination protocols, a proportion of primiparous sows might not sufficiently seroconvert to provide sufficient passive immunity to their offsprings. A simple adaptation of the vaccination scheme can significantly improve passive protection of piglets from *C. perfringens* type C enteritis.

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Conflict of interest: No

P 129

High-fat Diet Increases Severity of *C. difficile* Infection via the Microbiome

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Recent clinical studies have implicated obesity with increased susceptibility and severity of *Clostridioides* (*Clostridium*) *difficile* infection (CDI). Diet is a major driver of obesity in addition its role in shaping the structure and the function of the microbiota. Other groups have shown that low protein or high zinc diets alter susceptibility to CDI. Thus, we sought to test the role of a high-fat Western diet in susceptibility to CDI using a murine model of infection. Mice maintained on a high-fat, high-simple sugar Western-diet for four weeks gained significantly more weight compared to mice fed a low-fat control diet (28.1 ± 1.8 g vs 25.4 ± 2.1 g, p =0.003). Upon *C. difficile* infection , mice on the high-fat diet experienced more severe disease as measured by decreased weights and increased clinical score (17.6± 3.5 vs. 11.5 ± 3.9, p=0.002). To test if increased severity was due to diet-induced changes to the microbiome, mice were maintained on their respective diets, but soiled bedding was swapped between the groups. Surprisingly, mice fed a control diet but receiving bedding from mice on a Western-diet before infection experienced significantly worse disease, including increased clinical scores (12.0 ± 8.4 vs. 1.5 ± 2.7, p=0.001). Similarly, mice fed Western-diet but receiving bedding from control-diet fed mice were protected from severe disease as measured by clinical score (14.1± 5.6 vs. 8.8 ± .6, p=0.03). This suggests the microbiome, altered by the diet, drives differential susceptibility to CDI. Future work will leverage metagenomics and metabolomics to identify the functional changes in the microbiome which are responsible for diet-mediated altered susceptibility to CDI.

Conflict of interest: No

P 130

A sporulation signature thiol protease governs spore structure, germination and host colonization in *Clostridioides difficile*

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A genomic signature for endosporulation includes a gene coding for a protease, YabG, which in the model organism *Bacillus subtilis* is required for proper assembly of the spore coat. In both *B. subtilis* and in Clostridioides difficile, YabG is produced late during spore development under the control of the mother cell-specific regulator σK and associates with the spore surface layers. We show that YabG is a cysteine (thiol) protease with a papain-like catalytic triad formed by conserved residues Cys207, His161 and Asp162. YabG is activated through removal of an inhibitory pro-domain in trans, and has an auto-proteolytic activity that limits the levels of the protease in vivo. The activity of YabG is also required for the processing of spore coat proteins such as CotE with both peroxiredoxin and chitinase activity, and CspBA, which releases the CspB protease, required for proper spore germination. A catalytically-inactive *yabG* mutant forms spores with a misassembled coat and exosporium that are more permeable to lysozyme and impaired in germination and host colonization.
Strikingly, we show that the misassembly of the spore coat and exosporium results in part from the requirement for YabG for the expression of late σK-dependent genes. These include *cdeM*, coding for an abundant component of the exosporium. Thus, YabG also impinges onto the genetic program of the mother cell and does so most likely by eliminating a transcriptional repressor. This repressor appears to be SpoIIID, which binds to the *cdeM* promoter and is cleaved by YabG. Moreover, placing cdeM under the control of a SpoIIID-independent promoter restores expression of *cdeM* to *yabG* mutants and largely bypasses the requirement for YabG for CdeM assembly. Previous work has shown that in *B. subtilis*, σK sets in motion a negative regulatory loop that limits the levels of SpoIIID late in sporulation and allows for proper expression of cot genes. Since *B.*

subtilis SpoIIID is also cleaved by YabG, the role of the protease in the control of the mother cell transcriptional program appears to be conserved in this organism. Our study shows that YabG is a novel type of cysteine (thiol) protease required for proper assembly of the spore surface layers. This function is conserved across evolutionary distance and is exerted both through processing of structural and functional components of the spore surface layers as well as the temporal control of expression of some of those genes.

Conflict of interest: No

P 131

Gut microbiota in *Clostridium difficile* colonized and non-colonized hospitalized patients

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Clostridium (*Clostridioides*) *difficile* is typically associated with a disturbed gut microbiota. Multiple different host specific and environmental factors are known to be associated with specific microbial signatures in the gut. Hospitalization introduces numerous alterations to the normal lifestyle. Medication, stress and diet change all potentially influence microbiota composition. *C. difficile* could be detected in about 10 - 20 % of patients upon admission to the hospital and the colonization rate increases with the time of hospitalization. Gastrointestinal diseases, e.g. IBD (Inflammatory bowel diseases), are known to be associated with increased risk for *C. difficile* colonization and infection.
Here we have studied a cohort of 121 patients hospitalized in the Department for gastroenterology at University Medical Centre in Maribor (Slovenia). Patients were hospitalized for a variety of primary diagnoses and were in this study classified into IBD and non-IBD group. Fecal samples were collected, *C. difficile* was detected with the specific in-house qPCR, the composition of gut microbiota was determined with the amplicon sequencing targeting V3V4 variable region of the 16S rRNA gene for bacterial and ITS2 region for the fungal community analysis.
C. difficile was detected in 41/121 (33,9 %) of all hospitalized patients. There was no significant difference in the colonization rate between non-IBD group (27; 33,3 %) and IBD group (14; 35 %).
In the IBD group no significant differences in either bacterial or fungal gut microbiota were observed between *C. difficile* colonized and non-colonized patients.
In the non-IBD group of hospitalized gastroenterological patients fungal microbiota also did not show any differences, while bacterial microbiota differed in diversity and composition between *C. difficile* colonized and non-colonized individuals.
In conclusion, all gastrointestinal patients and not only IBD patients are commonly colonized with *C. difficile*. The colonization was associated with different gut microbiota signatures onlyin non-IBD patients but not in IBD patients.

Conflict of interest: No

P 132

Elucidating how the putative *Clostridioides difficile* germinant receptor, CspC, integrates distinct co-germinant signals

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Clostridioides difficile is a Gram-positive anaerobic bacterium that causes approximately 500,000 gastrointestinal infections and nearly 30,000 deaths each year in the U.S., generating over $4 billion in excess medical costs annually. Formation of aerotolerant spores and subsequent germination of these spores into toxin-producing vegetative cells in the gut is critical for *C. difficile* to cause disease. *C. difficile* spores germinate in response to specific bile acids in the mammalian gut, and the CspC pseudoprotease was previously identified as the receptor for these bile acid germinants. However, recent work from our lab challenges the evidence implicating CspC as the germinant receptor. Our crystal structure-guided mutational analyses indicate that CspC integrates signals from not only bile acid germinants but also from two classes of co-germinants: amino acids and divalent cations. Specifically, we found that a D429K substitution in CspC increased the sensitivity of spores to calcium, while an R456G substitution increased sensitivity to amino acids. Since the mechanism by which CspC integrates these signals remains unknown, we tested whether CspC directly interacts with co-germinants. In thermal shift assays, we detected CspC binding to calcium but not to amino acids. Interestingly, the D429K substitution does not increase the affinity of CspC for calcium, suggesting that it transduces the calcium signal rather than directly interacting with calcium. To gain insight into signal conveyance through CspC we used intra-protein epistasis analyses and assessed the how the nature of the residues at positions 429 and 456 influences sensitivity to calcium and amino acids, respectively. These mutant analyses provide insight into the inter- or intra-protein conduits through which co-germinants signal in *C. difficile* spores.

Conflict of interest: No

P 133

Modified CodY in *C. difficile* strains

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Clostridium (Clostridioides) difficile is an important nosocomial pathogen which has been classified as "urgent threat" by CDC. Antibiotic use is the primary risk factor for the development of *C. difficile*- associated disease because it disrupts healthy protective gut flora and enables *C. difficile* to colonize the colon. *C. difficile* damage host tissue by secreting toxins and disseminates by forming spores. Nutrient availability and other environmental factors greatly influence toxin production in *C. difficile*. CodY, a protein found in many Gram-positive bacterial pathogens, is an important factor that regulates major metabolic pathways and virulence gene expression in response to nutrient availability. In *C. difficile* CodY represses toxin production and sporulation. We have identified that the codY gene in an epidemic *C. difficile* strain (R20291) carries a point mutation that changes its Tyrosine residue at the 146th position to an Asparagine (codYY146N). Using biochemical and genetic experiments, here we demonstrate that CodYY146N is less active than the wild-type CodY (CodYWT). Our search in the database following this analysis leads us to the CodYV58A allele in 20 different *C. difficile* strains of different origin. Similar to Y146 (at the dimer interface), V58 is located (ILV binding domain) at a crucial position in CodY and can influence its activity. We are currently testing the hypothesis that producing a less active CodY would significantly modify the virulence gene regulatory network.

Conflict of interest: No

P 134

High-resolution 16S sequencing and analysis of *C. difficile* colonization in a hospitalized elderly population

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Clostridioides difficile infection is the most common form of Antibiotic-Associated Diarrhea (AAD) affecting 500,000 patients yearly and causing 29,000 deaths in the United States. One of the primary risk factors associated with *C. difficile* infection (CDI) is colonization with *C. difficile* (CD). 16S sequencing is a sensitive method of detection of colonization and allows in-depth analysis of the intestinal bacterial community which could therefore increase our understanding of CD colonization and its relations to other organisms present in the gut. Here we show that 16S sequencing and analysis using oligotyping[1] enables CD detection in a hospitalized, European population receiving broad-spectrum antibiotics (N = 1007). Two samples were collected, one at baseline (D1, n = 945) and after treatment with broad-spectrum antibiotics (D6, n = 737), and patients were followed up during a 90-day period. A baseline CD colonization prevalence of 5.40% (n = 51) was observed with an observed CDI incidence of 7.84% (n = 4, 95% CI: 3.06-20.1%) during the study period, whereas the non-colonized study population showed a CDI incidence of 1.11% (n = 10, 95% CI: 0.60-2.07%). No difference in alpha diversity was seen between colonized and non-colonized patients, but a difference in microbial composition was observed (AMOVA, $p < 0.001$) where colonized microbiome profiles showed higher abundance of *Clostridium* clusters XI, XIVa, and XIII. Treatment with broad-spectrum antibiotics led to an increase in CD colonization prevalence at D6 (6.78%). Interestingly, only one OTU was classified as *Clostridium* XI, containing CD, but oligotyping showed that this OTU comprised of both CD as well as *Clostridium bartlettii*, another occupant of the human gut[2] which has a high sequence similarity, when using the standard clustering threshold of 97%. Our results show that CD colonized patients are at a higher risk of developing CDI compared to the overall population, and that 16S sequencing is a promising technology that could be developed into a screening tool to detect CD colonization in a high-risk hospitalized population a useful tool when studying colonization in a hospitalized population to better define the colonization concept.

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Conflict of interest: No

P 135

Production, isolation and interaction of botulinum neurotoxin E and its associated proteins from *C. botulinum* strain Beluga

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Botulinum neurotoxins causing the fatal disease botulism are classified into ten serotypes (BoNT/A to H, J, X) and are encoded in ha- or orfx-gene clusters of *C. botulinum* together with a non-toxic non-hemagglutinin (NTNHA). NTNHA and BoNT form a medium progenitor toxin complex (M-PTC) to survive degradation in the GI tract. Three hemagglutinins (HA1-3) encoded in ha-gene clusters bind to the M-PTC thereby increasing intestinal absorption of the neurotoxin. In contrast, the physiological and structural function of four proteins (OrfX1-3 and P 47) encoded in orfx-clusters of serotypes E, F, H, J, X and some serotype A subtypes remains unclear. The aim of our study is to examine expression profiles and to identify interactions of OrfX1-3 and P 47 with BoNT/E or NTNHA/E produced by *C. botulinum* strain Beluga.
C. botulinum strain Beluga was cultured and OrfX1-3 proteins as well as M-PTC/E were isolated by different means. Possible interactions between BoNT/E, NTNHA/E and OrfX1-3 were investigated by size exclusion chromatography and immunoprecipitation studies with various monoclonal antibodies specific for BoNT/E, NTNHA and OrfX1-3 as well as GST-fused anti-BoNT/E nanobodies.
All OrfX1-3 proteins as well as BoNT/E and NTNHA/E could be simultaneously extracted from *C. botulinum* cell pellets and were detected by Western Blotting. Proteins were immunoprecipitated by their respective monoclonal antibodies/nanobodies. At pH 6.0, NTNHA/E was co-immunoprecipitated by BoNT/E and vice versa from cell pellet extracts while hardly any M-PTC/E was detectable in the culture supernantant. At neutral pH, the M-PTC/E was dissociated. Furthermore, OrfX2 was immunoprecipitated by M-PTC/E at pH 6.0. At neutral PH, the immunoprecipitation of OrfX2 could be ascribed to an interaction with NTNHA/E. To understand the mechanism of oral intoxication by BoNT/E from type II *C.*

botulinum, knowledge about its production and release together with non-toxic neurotoxin associated proteins is crucial. We show that M-PTC/E as well as OrfX1-3 produced by the *C. botulinum* group II strain Beluga could be extracted. For the first time, a direct interaction between an OrfX protein and NTNHA was demonstrated suggesting that OrfX2 facilitates either BoNT release from *C. botulinum* or intestinal absorption of BoNT upon intoxication.

Conflict of interest: No

P 136

Holin-dependent Secretion of the Large Clostridial Toxin TpeL from *Clostridium perfringens*

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The *C. perfringens* large clostridial toxin TpeL is in the family of toxins that include the *C. difficile* toxins TcdA and TcdB, as well as the *C. sordellii* TcsL and TcsH toxins. In each of these species, a gene encoding a holin-like protein has been identified in each genetic locus containing the toxins. For *C. difficile*, it has been demonstrated that the holin-like protein TcdE is required for secretion of TcdA and TcdB (1, 2). The holin-like protein associated with TpeL, TpeE, differs from TcdE in being a much smaller protein and having just a single transmembrane domain, making TpeE more similar to pinholins than true holins. In order to determine what the minimal requirements for TpeL secretion were, we cloned the tpeL and tpeE genes from a type C strain of *C. perfringens* and expressed them in the genetically malleable strain, HN13 (a derivative of strain 13). Secretion of TpeL required TpeE to be expressed as well, indicating not only that TpeE was required for secretion, but no other type C strain-specific proteins were involved. Our hypothesis is that the holin-like proteins form pores in the membrane which allow passage of the folded toxins from the cytoplasm to the space between the membrane and peptidoglycan. In support of this hypothesis, expression of TpeE alone in *E. coli* was toxic, but co-expression with the TpeL toxin relieved the toxicity, suggesting TpeL may act as a plug for the pore formed by the holin. In contrast, expression of TpeE alone in *C. perfringens* was not toxic, suggesting TpeE has a different effect on the membranes of *C. perfringens* and *E. coli*. Current studies to identify domains in both TpeE and TpeL that are needed for secretion are in progress and may help us develop a model for holin-dependent toxin secretion in the Clostridia.

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2. Govind R, Dupuy B. 2012. Secretion of *Clostridium difficile* toxins A and B requires the holin-like protein TcdE. PLoS Pathog 8:e1002727.

Conflict of interest: No

P 137

Phenotypic and Molecular antimicrobial susceptibility of Mexican *Clostridium difficile* strains

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Clostridium difficile is a major cause of antibiotic-associated diarrhea, the incidence and severity of the disease is increasing but treatment is not always effective, new disease treatment options are needed(1).
To determine the patterns of antimicrobial resistance in *C. difficile* isolated from Mexican patients with nosocomial diarrhea and to analyze by sequencing the molecular mechanisms of resistance.
Methods: 244 stool samples were obtained (173 adults and 71 children) in four hospitals of Mexico, City. We used macrodilution method and E-test for to determine antimicrobial susceptibility on 60 *C. difficile* isolates. Metronidazole, tigecycline, vancomycin, clindamcyn, ciprofloxacine, tetracycline, moxifloxacine, levofloxacine , chloramphenicol, linezolind, fusidic acid and florfenicol were tested. Twenty-seven isolates were sequenced by means of the "illumina" platform and MLST analysis was performed.
22 *C. difficile* strains(NAP1/027) belonged to clade 2 where the epidemic and hypertoxigenic strains are grouped, 2 strains belonging to clade 1,two isolates belonging to the clade 4 and only one belong to clade C-1. All strains of *C. difficile* were susceptible to vancomycin and metronidazole and demostrated high rate of resistance and elevated MICs to ciprofloxacin, moxifloxacin levofloxacin, rifampicin, linezolid, and clindamycin. Resistance to more three antibiotics were observed in 45 isolates. The results indicated that 100%(27/27) *C. difficile* strains had mutations in *gyrA*, *gyrB*, *rpoB* genes.

This study established the high degree of resistance and genetic diversity in Mexican *C. difficile* isolates
1. Spigaglia P. 2016. Recent advances in the understanding of antibiotic resistance in *Clostridium difficile* infection. Ther Adv Infect Dis 3:23–42.

Conflict of interest: No

P 138

The C-terminal domain of *Clostridium difficile* TcdC is exposed on the bacterial surface

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Clostridium difficile is an opportunistic gut pathogen and the main cause of antibiotic-associated diarrhea. Symptoms of *C. difficile* infection can range from mild diarrhea to pseudomembranous colitis. The severity of CDI has been linked to the production of the main virulent factors, Toxin A and Toxin B. The toxins are encoded within the pathogenicity locus (PaLoc) that also contains the toxin positive regulator sigma factor TcdR and TcdC, a putative anti-sigma factor. TcdC function has not yet been fully understood. Although TcdC is proposed as an anti-sigma factor, several studies lack to show association between TcdC genotype and toxin production. Previous studies have characterized TcdC as a membrane associated protein with the ability to bind G-quadruplex structures [1,2]. The binding to the DNA secondary structures is mediated through the OB-fold domain present at the C-terminus of the protein, a domain previously also proposed to be responsible for the inhibitory effect on toxin production [3].
In this study we aimed to determine TcdC topology. TcdC is predicted to have an N-terminal hydrophobic domain for membrane localization and a C-terminus active domain located extracellularly (predicted by TMHMM, http://www.cbs.dtu.dk/services/TMHMM/), which would contradict the cytoplasmic anti-sigma factor activity proposed previously Using Scanning Cysteine Accessibility Mutagenesis and the codon-optimized HiBiT luciferase system we were able to demonstrate that the TcdC C-terminus is located extracellularly as predicted. The extracellular location of TcdC does not support the binding of the active OB-fold domain to intracellular targets, limiting its role as an anti-sigma factor. Further studies are required to elucidate the role of TcdC.

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Conflict of interest: No

P 140

Clostridioides difficile LuxS mediates inter-bacterial interactions within biofilms

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The anaerobic gut pathogen, *Clostridioides difficile*, forms adherent biofilms that may play an important role in recurrent *C. difficile* infections. The mechanisms underlying *C. difficile* community formation and inter-bacterial interactions are nevertheless poorly understood. *C. difficile* produces AI-2, a quorum sensing molecule that modulates biofilm formation across many bacterial species. We found that a strain defective in LuxS, the enzyme that mediates AI-2 production, is defective in biofilm development *in vitro*. Transcriptomic analyses of biofilms formed by wild type (WT) and *luxS* mutant (*luxS*) strains revealed a downregulation of prophage loci in the *luxS* mutant biofilms compared to the WT. Detection of phages and eDNA within biofilms may suggest that DNA release by phage-mediated cell lysis contributes to *C. difficile* biofilm formation. In order to understand if LuxS mediates *C. difficile* crosstalk with other gut species, *C. difficile* interactions with a common gut bacterium, *Bacteroides fragilis*, were studied. We demonstrate that *C. difficile* growth is significantly reduced when co-cultured with *B. fragilis* in mixed biofilms. Interestingly, the absence of *C. difficile* LuxS alleviates the *B. fragilis*-mediated growth inhibition. Dual species RNA-sequencing analyses from single and mixed biofilms revealed differential modulation of distinct metabolic pathways for *C. difficile* WT, *luxS* and *B. fragilis* upon co-culture, indicating that AI-2 may be involved in induction of selective metabolic responses in *B. fragilis*. Overall, our data suggest that *C. difficile* LuxS/AI-2 utilises different mechanisms to mediate formation of single and mixed species communities.

Conflict of interest: No

P 141

An *in silico* survey of *Clostridioides difficile* extrachromosomal elements

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The gram-positive enteropathogen *Clostridioides difficile* is the major cause of healthcare associated diarrhoea and is also an important cause of community-acquired infectious diarrhoea. Considering the burden of the disease, many studies have employed whole genome sequencing to identify factors that contribute to virulence and pathogenesis. Though extrachromosomal elements such as plasmids are important for these processes in other bacteria, the few characterized plasmids of *C. difficile* have no relevant functions assigned and no systematic identification of plasmids has been carried out to date. Here, we perform an in silico analysis of publicly available sequence data, to show that ~13% of all *C. difficile* strains contain extrachromosomal elements, with 1-6 elements per strain. Our approach identifies known plasmids (e.g. pCD6, pCD630 and cloning plasmids) and 6 novel putative plasmid families. Our study shows that plasmids are abundant and may encode functions that are relevant for *C. difficile* physiology. The newly identified plasmids may also form the basis for the construction of novel cloning plasmids for *C. difficile* that are compatible with existing tools.

Conflict of interest: No

P 142

Experiences in *Clostridiodes difficile* treatment of the Netherlands Donor Feces Bank: the power of consultation by the NDFB working group

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In recent years Fecal Microbiota Transplantation (FMT) is effectively implemented as an approved treatment approach of refractory *Clostridiodes difficile* infection (rCDI). The Netherlands Donor Feces Bank (NDFB) was founded to facilitate this treatment by providing ready to use donor feces suspension for treatment of patients. A working group of medical specialists assesses all the FMT requests, to ensure that the indication is correct, and to optimize the effectiveness of FMT. The NDFB is located at the Leiden University Medical Center (LUMC) and is supported by a working group of specialists in the fields of Medical Microbiology, Gastroenterology, Internal Medicine, Infectious Diseases, Biobanking and Methodology of different hospitals in the Netherlands. A request for FMT is evaluated by at least three clinical members of the NDFB working group to assess the indication of FMT, the approve the diagnosis rCDI, and to formulate a treatment advise for each individual patient taking the comorbidity into account. Since the start of NDFB in March 2016, 167 FMT requests to treat 153 (r) CDI patients were reviewed within the NDFB working group. In total, 133 FMTs were used for treatment of 120 patients, with recurrent CDI as the most common indication. A cure rate of 90.4% was observed in FMT treated rCDI patients. Of all the requests, 20.4% of the patients were deemed not suitable for FMT by the NDFB working group. The most common reason for rejection was carriership instead of infection of *C. difficile* in combination with diarrhoea due to another cause in 15/34 patients (inflammatory bowel disease, n=8; ischaemic colitis n=2; Graft-versus-Host-Disease, n=2; other reasons, n=4). Furthermore, FMT was rejected in 5 patients without true recurrent CDI, 4 patients without a convincing diagnosis of CDI. Postponement of FMT was advised in two patients because of long term antibiotic treatment for other diseases. In 2 patients, there was a combination of the above mentioned criteria.

To achieve effective and optimal use of FMT for patients with a recurrent *Clostridiodes difficile* infection, consultation by a multidisciplinary approach seems beneficial. This approach may explain the high cure rate of FMT with suspensions provided by the NDFB, and prevents unnecessary treatment of patients with FMT.

Conflict of interest: No

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The psychological effect of Fecal Microbiota Transplantation in patients with recurrent *Clostridium difficile* infections

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The gut-brain-axis is considered as a bidirectional communication system between the brain and the gut microbiota. A growing number of studies show the importance of gut microbiota in the pathogenesis and clinical course of neuropsychiatric diseases, but not much information is available of the effect Fecal Microbiota Transplantation (FMT).
Objectives: The aim of the study was to explore the psychological effect of FMT of healthy donors in patients with recurrent *Clostridium difficile* infections on short and long term.
Prior to FMT, three weeks and six months after FMT for recurrent *Clostridium difficile* infections (rCDI) treated patients completed a questionnaire about their self-rated health, happiness, dispositional optimism, and symptoms of anxiety and depression.
In total 51 patients were included prior to FMT. The mean age was 68,8 years (±14,7) and 34 patients were female. 33 patients (22 females) were included for short term follow up after FMT with a mean age of 68,0 years (±14,0). 18 patients (12 females) were already included for long term follow up after FMT with a mean age of 70,7 years (±13,8). Significant improvements in self-rated health and loss of interest were found, although improvements were explained by a decrease in somatic illness. No effect was found for cognitive emotional changes.
Some aspects of mental well-being in rCDI patients treated with FMT improve on short and long term. This can be ascribed to the resolution of somatic illness.

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Conflict of interest: No

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