

ABSTRACT BOOK

Contents:

Welcome Address	P. 2
General information	P. 3
Acknowledgments	P. 4
Program	P. 5
Opening lecture	P. 12
Clostridia in general, their toxins and other pathogenicity factors	P. 14
<i>C. difficile</i>	P. 24
Clostridia: clinical issues	P. 29

Welcome to Villars-sur-Ollon

On behalf of the Federation of European Microbiological Societies (FEMS) and the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), we have the privilege to introduce the *Fourth Conference on New Frontiers in Microbiology and Infection*. This is the continuation of a "venture" between the two major European organisations in the field.

These conferences are aimed at bringing together basic and clinical microbiologists as well as infectious diseases specialists. An additional purpose is to give young scientists all over the world the opportunity to interact with senior colleagues. These meetings are now being held on annual basis. They are intended to be outstanding events in the field of microbiology.

Clostridia, particularly *C. difficile*, are of relevant importance in the global field of infectious diseases. This is why we chose to focus on clostridial organisms for the forth conference. We are honoured that some of the best international scientists in the field of Clostridia accepted our invitation and would like to thank them for their commitment to the success of the meeting. We also would like to thank all the participants who showed interest in this conference by presenting their work or simply by participating to the discussions.

As a final remark, we wish to express our grateful thanks to Martine Moreillon who, with both enthusiasm and professionalism, has been greatly instrumental in organising this meeting within a few months.

Have a nice and fruitful stay in Villars!



Roland J. Koerner



Jean-Claude Piffaretti



Jordi Vila

Javier Garau Alemany

General Information: www.cnfmi.org

Organising Committee

Javier Garau, Barcelona, Spain
Roland Koerner, Sunderland, UK
Jean-Claude Piffaretti, Massagno, Switzerland
Jordi Vila, Barcelona, Spain

Scientific Committee

Axel T. Brunger, Stanford, US
David Dockrell, Sheffield, UK
Ed J. Kuijper, Leiden, NL
Michel-Robert Popoff, Paris, FR
Ian R. Poxton, Edinburgh, UK
Maja Rupnik, Maribor, SI

Conference Venue

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Acknowledgments

The organisation of the Conference has been possible thanks to the contributions of:

- The European Society of Clinical Microbiology and Infectious Diseases (**ESCMID**)

The European Society of Clinical Microbiology and Infectious Diseases is a non-profit organisation whose mission is to improve the diagnosis, treatment and prevention of infectious diseases by promoting and supporting research, education and training in the infection disciplines. This is achieved by scientific exchange, educational programmes, grants and awards, certification and consultation with professional and government agencies. Through its activities and publications ESCMID fosters professional exchange and stimulates an open and collaborative spirit nourished by the diversity of European culture.

www.escmid.org

- The Federation of European Microbiological Societies (**FEMS**)

The Federation of European Microbiological Societies is a non-profit organisation devoted to the promotion of microbiology in Europe. FEMS is currently linking 47 microbiological societies, encouraging joint activities, facilitating communication among microbiologists, supporting meetings and laboratory courses, providing fellowships, and publishing journals and books.

www.fems-microbiology.org

- The University of Lausanne **UNIL**

Scientific Program

Sunday, 5 October 2008

Opening lecture

18:30 The discovery of *Clostridium* and its clinical impact. *Roland Koerner, Sunderland Royal
Hospital, Sunderland, UK*

 An insight in the history of medicine

19:30 **Welcome Aperitif**

20:00 **Dinner**

Scientific Program

Monday, 6 October 2008

***Clostridia* in general, their toxins and other pathogenicity factors**

08:30 **Morning session: plenary lectures**

Chairs:

Michel-Robert Popoff, Paris, F
Axel T. Brunger, Stanford, USA

08:30 Basis of the mode of action of clostridial toxins

Michel-Robert Popoff,
Institut Pasteur, Paris, F

09 :30 Insights into the mechanism of botulinum neurotoxin (BoNT) receptor binding and substrate cleavage from a structural perspective

Axel T. Brunger, Howard Hughes
Medical Institute, Stanford, USA

10:30 **Coffee Break**

11:00 *C. perfringens* epsilon-toxin

Juan Blasi, University of Barcelona,
Barcelona, ES

12:00 Comparative genomics of clostridia and pathogenic properties

Holger Brüggemann,
Max Planck Institute for Infection
Biology, Berlin, DE

13:00 **Lunch**

Scientific Program

Monday, 6 October 2008

- 14:30 **Afternoon session: abstract presentations and discussion around the poster boards** **Chairs:**
Michel-Robert Popoff, Paris, F
Axel T. Brunger, Stanford, USA
- 14:30 Lytic phages against *Clostridium Perfringens* *Almas Begdullayev ,Scientific center for anti-infectious drugs"KazBioMed", Almaty, Kasakhstan*
- 14:50 Regulation of *Clostridium difficile* toxin expression *Jeroen Corver, Leiden University Medical Center, Leiden, NL*
- 15:10 Structure-function analysis of *Clostridium difficile* toxin E expressed in *Escherichia coli* *Alexandra Olling, Hannover Medical School, Hannover, DE*
- 15:30 Genotyping of *Clostridium difficile* in Finland, 2007-2008 *Saara Kotila, National Public Health Institute of Finland, Helsinki, FIN*
- 15:50 Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, PCR-ribotype 078 *Abraham Goorhuis, Leiden University Medical Center, Leiden, NL*
- 19:30 **Dinner**

Scientific Program

Tuesday, 7 October 2008

C. difficile

08:30	Morning session: plenary lectures	Chairs: <i>Maja Rupnik, Maribor, Slovenia</i> <i>Ed J. Kuijper, Leiden, NL</i>
08:30	<i>Clostridium difficile</i> : an overview of the changes in our understanding the organism over the last 30 years	<i>Maja Rupnik, Institute of Public Health, Maribor, SI</i>
09 :30	<i>C. difficile</i> – the wider perspective (humans, animals, environment)	<i>Viveca Båverud, National Veterinary Institute, Uppsala, SE</i>
10:30	Coffee Break	
11:00	<i>Clostridium difficile</i> : an overview of the disease, host defences, risk factors and changing host susceptibility	<i>Ian R. Poxton, Centre for Infectious Diseases, Edinburgh, UK</i>
12:00	Clinical spectrum of <i>Clostridium difficile</i> Infection (CDI) and the emergence of hypervirulent strains	<i>Ed J. Kuijper, Centre for Infectious Diseases, Leiden, NL</i>
13:00	Lunch	
14:00	Day Trip	
19:30	Dinner	

Scientific Program

Wednesday, 8 October 2008

Clostridia: clinical issues

08:30	Morning session: plenary lectures	Chairs: <i>David Dockrell, Sheffield, UK</i> <i>Ian R. Poxton, Edinburgh, UK</i>
08:30	Clostridial infections in the immunocompromised host	<i>David Dockrell, Royal Hallamshire Hospital, Sheffield, UK</i>
09 :30	Emerging clostridial infections in USA	<i>L. Clifford McDonald, Centers for Disease Control and Prevention, Atlanta, USA</i>
10:30	Coffee Break	
11:00	Clostridia in cancer therapy	<i>Jan Theys, University Maastricht, Maastricht, NL</i>
12:00	Toll-like receptors and intestinal inflammation	<i>William F. Stenson, Washington University School of Medicine, St-Louis, USA</i>
13:00	Lunch	

Scientific Program

Wednesday, 8 October 2008

Clostridia: clinical issues

14:30	Afternoon session: abstract presentations and discussion around the poster boards	Chairs: <i>David Dockrell, Sheffield, UK</i> <i>Ian R. Poxton, Edinburgh, UK</i>
14:30	Clinical-epidemiological characterization of <i>Clostridium difficile</i> -Ribotype 053 - a new strain with high transmissibility	<i>Markus Hell, University Hospital Salzburg, Salzburg, Austria</i>
14:50	Characterization of <i>Clostridium difficile</i> ribotype 078 from human and animal origin	<i>Dennis Bakker, Leiden University Medical Hospital, Leiden, NL</i>
15:10	Isolation of <i>Clostridium difficile</i> from domestic and wild avian species	<i>Mateja Zemljic, Institute of Public Health Maribor, Maribor, SI</i>
15:30	Clinical significance of clostridial bacteremia: a retrospective three-year analysis in a French University Hospital Center	<i>Alain Lozniewski, University Hospital Center of Nancy, Nancy, F</i>
15:50	Risk factors for <i>Clostridium difficile</i> infections in an endemic setting in The Netherlands	<i>Abraham Goorhuis, Leiden University Medical Center, Leiden, NL</i>
16:10	Coffee Break	
16:40	Round table and conclusion	Chair: <i>Roland Koerner, Sunderland Royal Hospital, Sunderland, UK</i>
19:30	Dinner	

4th Conference: Clostridia: from old diseases to new threats – basic science meets infectious diseases

Thursday, 9 October 2008

Bus leaving in front of the Eurotel Victoria to the Geneva-airport at 8:00 a.m

Opening Lecture

The discovery of Clostridium and its clinical impact: an insight in the history of medicine

Roland Koerner

Sunderland Royal Hospital, UK

The objective of this introductory lecture is to provide an overview of the historical background and the progress in the understanding of the pathogenesis of disease leading to accepting bacteria as the aetiological agents of gas gangrene, tetanus and botulism. The impact of the newly gained insight into the pathogenesis of infection on the debate about novel therapeutic approaches, which were seen as mutually exclusive, will be discussed:

Clostridium perfringens was described by Welch in 1892 (*Bacillus aerogenes capsulatus*) and by Fraenkel in 1893 (*Bacillus phlegmonis emphysematosae*). Fraenkel proved that this organism causes gas gangrene, uterine gangrene, septic abortion etc. During the First World War “gas-forming *Clostridium species*” turned out to be the main cause for the high mortality rate secondary to wound infections amongst wounded soldiers. The “misinterpretation” of new insights into the physiology of wound healing led to a less aggressive surgical approach. This in turn enabled spores of environmental *Clostridium spp* to germinate in the traumatized tissue and exert their full pathogenic potential. In the Second World War the combination of aggressive surgery combined with antimicrobial chemotherapy, first 4-aminomethyl-benzosulphonamide (Marfanil) and, once available, penicillin led to a substantially reduced infection-associated mortality amongst war casualties.

The earliest description of botulism as a clinical entity originates from Constantinople about 1000 years ago. At the turn of the 18th century Justinus Kerner described the classical clinical presentation of botulism. In 1896 Pierre van Ermengem reported the isolation of “*Bacillus botulinus*” as the aetiological agent of botulism. His work is a classical example how thorough epidemiological investigations combined with laboratory bacteriological analysis lead to the discovery of the tentative aetiological agent. Finally, the application of Koch’s postulates confirms the hypothesis.

The association of wounds and the subsequent development of fatal muscle spasms have been known to man since ancient times. The confirmation that tetanus is caused by an infective agent reads like success story of early bacteriology. In 1884, Nicolaier demonstrated the strychnine-like action of a toxin excreted by free-living, anaerobic soil bacteria and 1884 Carle and Rattone demonstrated the transmissibility of tetanus for the first time. In 1886 Flügge described “*Bacillus tetani*” In 1897, Nocard showed that tetanus antitoxin induced passive immunity in humans. In 1901 Bering and Kitasato introduced an anti-serum to tetanus. The toxoid vaccine was developed by P. Descombey in 1924, and is still used to prevent tetanus.

***Clostridia in general, their
toxins and other
pathogenicity factors***

Basic modes of action of clostridial toxins

M. R. Popoff

Bactéries anaérobies et Toxines, Institut Pasteur, Paris, France

Clostridia are the bacterial group which produces the largest number of toxins and the most potent toxins, responsible for severe diseases in man and animals. In a first step, toxins recognize a specific receptor on target cell surface. Some of these receptors are ubiquitous and thus the corresponding toxins are active on a large number of cell types, whereas other toxins interact with only certain cell types (neurotoxins, enterotoxins ...). Numerous clostridial toxins are active on cell membrane by pore formation (pore forming toxins, PFTs), or by an enzymatic activity (phospholipase) leading to a degradation of cell wall components and disruption of cell membrane integrity. PFTs share a common mechanism of action consisting in interaction with a cell surface receptor, oligomerization, structural change with unfolding of beta-strands forming a beta-barrel, and then insertion of the beta-barrel into the lipid bilayer leading to a pore formation. Three groups of clostridial toxins are active intracellularly: clostridial binary toxins, large clostridial toxins, and neurotoxins. Clostridial toxins are constituted of a binding component, which is structurally related to PFTs, and an enzymatic component. Binding components mediate the entry of the enzymatic component into cells through pore across the endosome membrane. Large clostridial toxins and neurotoxins are single chain proteins, which enter cells by receptor-mediated endocytosis. Large clostridial toxins release their enzymatic domain into the cytosol by an autoproteolytic process, whereas neurotoxins use a disulfide bond reduction. Clostridial binary toxins and large clostridial toxins alter the actin cytoskeleton by enzymatically modifying actin monomers or regulatory GTPases, respectively. Neurotoxins target specifically the exocytosis machinery in neuronal cells and thus inhibit the evoked release of neurotransmitters.

Clostridia in general, their toxins and other pathogenicity factors

Insights into the mechanism of botulinum neurotoxin (BoNT) receptor binding and substrate cleavage from a structural perspective

Axel T. Brunger

Department of Molecular and Cellular Physiology, Stanford University and Howard Hughes Medical Institute, Stanford, USA

The high potency of clostridial neurotoxins relies predominantly on their neurospecific binding and specific hydrolysis of SNARE proteins. Their multi step mode of mechanism can be ascribed to their multi-domain three-dimensional structure. The C-terminal H_{CC}-domain interacts subsequently with complex polysialo-gangliosides (such as GT1b) and a synaptic vesicle protein receptor (such as synaptotagmin or SV2C) via two neighbouring binding sites resulting in highly specific uptake of the neurotoxins at synapses of cholinergic motoneurons. After its translocation the enzymatically active light chain specifically hydrolyses specific neuronal SNARE proteins (synaptotabrevin, syntaxin, SNAP-25), preventing SNARE complex assembly and thereby blocking exocytosis of neurotransmitter. The specific interactions for receptor and substrate binding will be described and implications for the molecular mechanism of action of BoNTs discussed.

C. perfringens epsilon-toxin

Juan Blasi

Department of Pathology and Experimental Therapeutics. University of Barcelona-IDIBELL. C/ Feixa Llarga s/n. 08907 L'Hospitalet de Llobregat. Spain

Epsilon toxin (ϵ -toxin) is the most potent clostridial toxin after botulinum and tetanus neurotoxins. The toxin is produced by *Clostridium perfringens* types B and D and causes fatal enterotoxemia in sheep, goats and, occasionally calves and other animals, becoming in heavy economic losses. Disease is characterized by an increase in intestinal permeability, oedema of the brain, lung, heart and kidney. It is also known as sudden death, pulp kidney or overeating disease. Toxin is synthesized as an inactive prototoxin of 311 amino acids (32.7 kDa) which is converted to fully active toxin by proteolytic removal of a N- and C terminal peptides of 14 and 23 residues respectively.

Circulating ϵ -toxin specifically binds to renal epithelial distal tubule cells and to cerebral endothelia, where it may exert a direct effect by permeabilising the blood–brain barrier. In fact, the toxin causes neurological disorders with variable signs depending on the circulating toxin levels.

The Madin-Darby canine kidney (MDCK) cell line, of epithelial origin from the distal-collecting tubule, has been widely used as a cellular model for the study of the cellular and molecular mechanisms involved in ϵ -toxin activity. Using this model it has been reported, the correlation of the intoxication process with the formation of a membrane complex of about 155 kDa and the efflux of intracellular K^+ and the influx of Na^+ , Cl^- and Ca^{++} . This effect, together with a depletion of cellular ATP has been recently shown in renal collecting duct cells (Chassin et al., 2007, Am J Physiol Renal Physiol. 2007 293(3):F927-37).

Using recombinant ϵ -toxin–green fluorescence (ϵ -toxin–GFP) and ϵ -prototoxin–GFP fusion proteins we have studied the binding and potential cellular targets in mice, an animal model widely used for laboratory controlled intoxication studies. ϵ -Toxin–GFP binds in vivo to endothelial cells and renal tubules, where it has a strong cytotoxic effect. Those binding experiments indicate that an ϵ -toxin receptor might be expressed on renal distal tubules of mammalian species, including human, a fact that explain the high sensitivity of cell lines from distal-collecting renal tubules to the toxin. Binding experiments also showed specific binding of ϵ -toxin to either central and peripheral myelinic structures.

Direct fluorescence tracking of the toxin demonstrated the toxin's ability to bind to cerebral blood vessels inducing severe perivascular oedema, to cross the BBB and to accumulate in the brain parenchyma where binding to glial cells is also occasionally detected.

Further studies are still required to completely understand the ϵ -toxin intoxication pathway, including the existence of potential cellular receptors and its ability to cross the blood brain barrier.

Comparative genomics of pathogenic clostridia

Holger Brüggemann

*Max Planck Institute for Infection Biology, Department of Molecular Biology, Charitéplatz 1,
D-10117 Berlin, GERMANY*

Since the year 2000, the genomes of ten clostridial species have been completely sequenced, starting with the genome of the solvent-producing nonpathogenic *C. acetobutylicum* in 2001. Subsequently, the genomes of major clostridial pathogens were sequenced, including *C. perfringens*, *C. tetani*, *C. novyi*, *C. botulinum*, and *C. difficile*. The genomes gave us insights in terms of their plasticity and evolution. In addition, it became apparent that many clostridial toxin genes can be found on (or are closely associated with) mobile genetic elements, thus shedding light on questions regarding their distribution and possible horizontal acquisition.

More recently, genome sequencing has been applied to deduce the “pan-genome” of a given species: several different strains of *C. perfringens*, *C. botulinum* and *C. difficile* were and are being completely sequenced, which makes it possible to distinguish the ‘core’ genome from strain-specific genomic variation within a given species. The massive sequencing efforts also enabled large-scale transcriptomic and proteomic projects as well as functional studies on single gene level (thereby applying a recently developed gene knock-out technique).

This presentation will give an overview on finished and ongoing genome projects, and will introduce the concept of “pan-genomics”. Results of comparative genome studies will be presented and discussed, using *C. botulinum* as an example. Easy-to-use software tools for (comparative) genome analysis will be introduced as well, since genome analysis is not restricted to bioinformaticians any more. The second part of the presentation is devoted to specific biological features (such as metabolism, sporulation, cell surface and regulation) as deduced from genome analyses, thereby highlighting similarities and differences between the major clostridial pathogens.

Clostridia in general, their toxins and other pathogenicity factors

Lytic phages against Clostridium Perfringens

Begdullayev Almas

Scientific center for anti-infectious drugs, "KazBioMed", Almaty, Kasakhstan

We have isolated 44 different lytic bacteriophage active against 31 bacterial isolates of a 48 member *C. perfringens* library. The other 17 isolates have, to date, proven recalcitrant to lytic activity from any of the isolated bacteriophage and from any wild type phages enriched from the aforementioned sources. Neither mitomycin C nor UV irradiation induced lysis from potential lysogenic phage integrated in these bacterial strains. For 15 of the 17 phage, the isolated viruses showed a high degree of specificity, being lytic against only a single *C. perfringens* strain. In two cases, fCp42 and fCp49, phage were lytic against 6 and 9 different *C. perfringens* strains, respectively. Plaque morphology was highly variable ranging from completely clear pinpoint (<1 mm diam.) plaques on a background lawn, to gauze-web appearances and large (2-4 mm) clear rough edged plaques. By electron microscopy, negatively stained concentrated phage preparations included tailed structures with prominent head features. All of the phage examined were chloroform resistant.

Regulation of Clostridium difficile toxin expression

Jeroen Corver¹, Dennis Bakker¹, Ed Kuijper¹

*Leiden University Medical Center*¹, Leiden, NL

BACKGROUND: *Clostridium difficile* is the main cause of nosocomial diarrhoea. Well known virulence factors for *C. difficile* are the presence of the two large clostridial toxins, TcdA and TcdB and the binary toxin. In addition, high virulence is associated with deleterious mutations in the gene coding for the negative regulator of toxin expression, TcdC. Toxin expression is positively regulated by the sigma factor TcdR, which is essential for recognition of tcdA and tcdB promoters by the RNA polymerase. TcdC has been described to act as an anti-sigma factor, which means that it prevents toxin promoter recognition by the RNA polymerase/TcdR complex, and thus is responsible for inhibition of transcription of the toxin genes.

METHODS: To investigate the biochemical properties of TcdC, TcdC and mutants thereof were expressed in *E.coli* and purified. Mutants had deletions of the hydrophobic N-terminus, the proposed dimerization domain or the C-terminus. The dimerization of the mutants was compared to wild type TcdC using SDS-PAGE and gel filtration.

RESULTS: TcdC formed dimers and multimers. By deletion of the putative dimerization domain of TcdC, the dimerization of TcdC was indeed strongly impaired. Other mutations (N-or C-terminus) did not result in alteration of the dimerization.

CONCLUSIONS: TcdC dimerizes through a coiled-coil domain, as was previously suggested.

Clostridia in general, their toxins and other pathogenicity factors

Structure-function analysis of Clostridium difficile toxin E expressed in Escherichia coli

Alexandra Olling¹, Sophie Seehase¹, Helma Tatge¹, Ingo Just¹, Ralf Gerhard¹

Hannover Medical School¹, Hannover, DE

BACKGROUND: The pathogenicity locus of *Clostridium difficile* exhibits an ORF (tcdE) located between the tcdA and tcdB genes encoding the 19 kDa protein toxin E (TcdE) which shows structural similarities to bacteriophage-encoded holins. This finding led to the hypothesis that TcdE causes cell lysis of *C. difficile* to facilitate release of the pathogenic factors toxins A and B.

METHODS: Since expression of TcdE in *E.coli* leads to bacterial cell lysis, growth profiles of *E.coli* expressing full length TcdE or deletion mutants were monitored. Additionally, site-directed mutagenesis was performed to investigate a putative dual-start-motif which might account for TcdE protein of different length and function. For specific detection western blot analysis with anti-TcdE serum was performed.

RESULTS: The expression of deletion mutants lacking either the N- or C- terminus or both resulted in inhibition of bacterial growth whereas a fusion construct of only the N- and C- termini had no effect. The expression pattern of wildtype TcdE reflects a 19kDa and a 16kDa protein. Mutagenesis analysis confirmed a dual start motif enabling the expression of proteins with different length and function, as it is known for holins. The mutation within the ribosome binding site shifted the ratio of the full length to the truncated protein from 1:10 to 1:1 accompanied by prolonged growth inhibition.

CONCLUSION: The hydrophobic transmembrane domains are essential for TcdE-induced inhibition of bacterial growth. The inhibitory effect is modulated by the ratio of longer (19kDa) to shorter (16kDa) protein which is regulated by a dual-start-motif.

GENOTYPING OF CLOSTRIDIUM DIFFICILE IN FINLAND, 2007-2008

Saara Kotila¹, Eija Könönen², Outi Lyytikäinen³, Salha Ibrahim², Silja Mentula², Anni Virolainen²

*National Public Health Institute of Finland*¹, *Department of Bacterial and Inflammatory Diseases, National Public Health Institute of Finland*², *Department of Infectious Disease Epidemiology, National Public Health Institute of Finland*³, Helsinki, FIN

Background: In October 2007, the first case of *Clostridium difficile*-associated disease (CDAD) caused by the hypervirulent PCR ribotype 027 was reported in Finland (Lyytikäinen et alii, 2007). Toxin positive CDAD became a notifiable disease from January 2008 onwards, and all clinical microbiology laboratories were asked to send *C. difficile* isolates from severe cases (according to the ECDC definition) and/or suspected outbreaks to the National Public Health Institute (KTL) for genotyping.

Materials and methods: During October 2007-August 2008, a total of 343 isolates from 11 out of the 20 health care districts were sent to the Respiratory and Anaerobic Bacteria Laboratory, KTL, to be genotyped. PCR ribotyping was performed according to the protocol of the Anaerobe Reference Unit in Cardiff, using their PCR ribotypes 001 and 027 as the reference. When a local outbreak was suspected, also pulsed-field gel electrophoresis (PFGE) was performed as previously described for *C. difficile* (Sawabe et alii, 2007) with some modifications. PCR ribotype and PFGE patterns were analyzed by the BioNumerics software (Applied Maths NV, Belgium).

Results: Of the 343 isolates, 163 (48%) were identified as the PCR ribotype 027 and 57 (17%) as the PCR ribotype 001; 123 (36%) isolates represented other ribotypes, among which over 40 distinct PCR ribotype profiles were identified. The isolates of *C. difficile* ribotype 027 came from 5 out of the 11 health care districts that had sent isolates to KTL, and originated from over 30 different health care facilities - most of them providing primary or long term care - located in southern and south-western Finland. All except 13 of the 163 subjects positive for the PCR ribotype 027 were 60 years of age or older. The non-027 ribotypes seemed to be equally common among severe cases as the 027 ribotypes. Thus far, PFGE has discriminated the isolates no further than PCR ribotyping.

Conclusion: In Finland, the hypervirulent *C. difficile* PCR ribotype 027, which was the dominating ribotype among the tested isolates, has spread at least in southern and south-western parts of the country. The impact of other ribotypes needs to be elucidated. However, eagerness of sending isolates to be typed varies between districts, thus hampering the epidemiological monitoring at the national level.

Emergence of Clostridium difficile infection due to a new hypervirulent strain, PCR-ribotype 078

Abraham Goorhuis¹, Dennis Bakker², Jeroen Corver², Sylvia Debast³, Celine Harmanus², Daan Notermans⁴, Aldert Bergwerff⁵, Friedo Dekker⁶, Kuijper Ed²

Leiden University Medical Center¹, Leiden University Medical Center, Department of Medical Microbiology, Leiden², Meander Medical Center, Department of Medical Microbiology, Amersfoort³, Center for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven⁴, University of Utrecht, Department of Veterinary Medicine, Utrecht⁵, Leiden University Medical Center, Department of Clinical Epidemiology, Leiden⁶, NL

Background

At the National Reference Laboratory in The Netherlands, an increase of *Clostridium difficile* Infections (CDI) due to PCR-ribotype 078 (Type 078) was noticed since 2005. This strain is predominant in cattle.

Methods

Clinical CDI caused by Type 078 was studied in relation to CDI caused by the hypervirulent Type 027 and by other Types than 027 or 078. All received human and porcine isolates were investigated by PCR ribotyping, toxinotyping, toxin gene analysis, sequencing of the toxin regulator gene *tcdC* and Multiple Locus Variable Number of Tandem Repeat Analysis (MLVA).

Results

Between February 2005 and February 2008, isolates from 1687 patients were investigated. Type 078 increased from 3% to 13% in this period. Compared to 027-patients, 078-patients were younger (67.4 vs. 73.5 years, $P < 0,01$) and more often had community-associated disease (17.5% vs. 6.7%, OR 2.98, 95%CI 2.11-8.02). Proportions of severe diarrhea (38.9% vs. 40.0%) and attributable mortality were similar (3.8% vs. 4.0%). Compared to patients with other types, 078-patients used more fluoroquinolones (29.4% vs. 19.8%; OR 2.17, 95%CI 1.06-4.44). Type 078 isolates contained *tcdA*, *tcdB*, binary toxin genes and a 39 bp deletion in *tcdC* as well as a point mutation at position 184, producing a stop codon. MLVA of 54 human and 11 porcine isolates showed 4 clonal complexes, containing both porcine and human isolates.

Conclusions

Type 078 is emerging in The Netherlands, with similar virulence markers and clinical disease as Type 027, affecting a younger population with more often community-associated disease. Human and porcine 078-isolates are genetically indistinguishable.

C. difficile

Clostridium difficile: an overview of the changes in our understanding the organism over the last 30 years

Maja Rupnik

Institute of Public Health Maribor and University of Maribor, Faculty of Medicine, Maribor, Slovenia

Clostridium difficile was recognized as the main cause of pseudomembranous colitis in 1978. Since then a lot of clinical and basic research has resulted into a better understanding of its epidemiology and pathogenesis and has at the same time revealed two completely new molecular mechanisms related to interaction of toxins with the host cell.

Virulence factors and pathogenesis. Two large toxins, toxin A (TcdA, enterotoxin) and toxin B (TcdB, cytotoxin) were recognized very early as the main virulence factors. In 90-ties the novel molecular mechanism of bacterial toxin activity – glucosyl transferase activity – was described for both of them. In 2007 additional novel mechanism – self cleavage activity – was found in *C. difficile* toxins. Our understanding of how TcdA and TcdB are causing the disease has changed over the last few years. First experiments on animal model have indicated synergistic action of both toxins and primary role of TcdA. Emergence of TcdA-negative, TcdB-positive strains with full virulence potential for humans changed this view. Recent results of toxin knock-out mutants again indicated that TcdB could have more important role. Additionally, the toxins could have tropism to other tissues than intestine.

Region coding for both toxins (pathogenicity locus, PaLoc) is well characterized and was found to be highly variable resulting in description of toxinotypes. In toxinotypes with significantly changed toxin genes another toxin, binary toxin CDT, is often present. Although binary toxin CDT was described already in 1988 its role in the pathogenesis still unclear.

Additional virulence factors (adhesion factors, sporulation potential, antibiotic resistance) are gaining more attention in last years.

Epidemiology. The dogma that *C. difficile* is a disease of elderly people after antibiotic therapy with mainly nosocomial onset is slowly changing. There is an increase in community-associated cases (although many of them have direct or indirect contact with hospital environment) and an increase in the populations with previously low risk. *C. difficile* is an emerging pathogen of animals, particularly pigs and calves.

The worldwide emergence of highly virulent types has influenced new developments of typing systems aiming at better discrimination and better interlaboratory comparability.

Diagnostics. Understanding of pathogenesis and epidemiology has influenced also the laboratory diagnostics. The cell culture cytotoxicity of fecal sample was long recognized as a gold standard, but was soon replaced by commercial immunological tests. These were first detecting TcdA only but has afterward changed to TcdA and TcdB specificity. Bacterial culture is gaining the importance. Molecular tests are being developed and after becoming commercially available they are likely to replace the toxin tests.

C. difficile

Clostridium difficile, the wider perspective (humans, animals, environment)

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Clostridium difficile is a well-known nosocomial pathogen in humans with antibiotic associated diarrhea. In the last decade, *C. difficile* has emerged as a pathogen also in animals. In horses, the organism and its toxins has demonstrated to be associated with antibiotic-associated diarrhea. Often, the horses have contracted the diarrhea at an animal hospital while being treated for diseases other than gastrointestinal. Despite intensive therapy, mortality has been high. *C. difficile* has also been associated as community associated diarrhea. When the diarrhea is antibiotic-associated it is of great importance to immediately discontinue the antibiotic treatment. When treatment is necessary the first-line antibiotic should be metronidazole.

Interestingly, *C. difficile* was associated with acute colitis in mares when their foals were treated orally with erythromycin in combination with rifampicin for *Rhodococcus equi* pneumonia. Some of the dams developed an acute fatal diarrhea due to accidental intake of small amounts of erythromycin and disturbance of the intestinal flora. Erythromycin was demonstrated to be a risk antibiotic in mature horses. When treating foals for *R. equi* pneumonia, it is important to avoid accidental ingestion of erythromycin by the dams.

In about 30% of healthy young foals up to 14 days of age, toxigenic *C. difficile* has been found in the normal gut flora. This is analogous with human infants and dog neonates who also have a high carrier rate.

To control infection, judicious use of antimicrobials is important. Infected horses must be isolated and routine examination of horses with antibiotic-associated diarrhea should be performed. To reduce the number of environmental spores, thorough cleaning and surface disinfection of the animal hospital and clinic are important. Use of disposable gloves and routine hand washing should be performed by all staff.

In the presentation, *C. difficile* in other animal species than horses will be discussed. In recent years, there have been reports in humans about a more virulent *C. difficile* ribotype 027/NAPI (North American pulsed field gel electrophoresis). PCR ribotyping of calf isolates demonstrated that ribotype 027 could be found in calves. A high frequency of *C. difficile* spores in retail meat products from beef has been found suggesting that food could be involved in the transmission of *C. difficile* from animals to humans.

In environmental studies, *C. difficile* or its spores can be isolated from the human hospital environment of patients with antibiotic-associated diarrhea. *C. difficile* has also been found at indoor surface samples at both large and small animal clinics in several countries. In addition, the microorganism was present in soil samples at stud farms but very rarely in soil samples at farms with only adult horses. Samples from e.g. soil, river waters, lake waters and swimming pools have been found positive in culture for *C. difficile*.

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Clostridium difficile: an overview of the disease it causes, host defences, risk factors and changing host susceptibility

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Clostridium difficile infections (CDI) range from mild, self-limiting diarrhoea, through to severe diarrhoea with pseudomembrane formation, and life-threatening fulminant colitis. The outcome of acquisition and colonisation of the bacterium is dependent on both the virulence of the organism and the susceptibility of the host. However, the symptoms are largely independent of the strain causing the disease, as the same strain can bring about varying effects in each infected host. Although uncommon in most healthy individuals, *C. difficile* is carried asymptotically by many hospitalised elderly as well as the neonate. This presentation will describe the pathogenesis of the disease primarily from the point of view of the host, investigating its responses to the pathogen and its toxins – highlighting what little we know and how much still needs to be learned.

The symptoms of the disease are a result of one or both of the two major virulence factors of the organism (toxins A and/or B) exerting their effects on the colonic mucosa. The insult is two fold: i) the cytotoxic properties of the toxins damage the colonocytes, and ii) action of the toxins in the lamina propria result in a possibly intense inflammatory response, with induction of pro-inflammatory cytokines and recruitment of neutrophils. These combined actions result in the loss of fluid by leakage through the damaged mucosa, reduced absorption and the formation of pseudomembranes following the “explosion” of neutrophils through the damaged mucosa and opened tight junctions with accumulations of fibrin.

Patient susceptibility may be due to several different (risk) factors. These include:

- The degree of disruption of the normally protective colonic microbiota and subsequent loss of colonisation resistance following action of antibiotics and other agents that might affect the gut ecosystem, including perhaps co-infection with another GI pathogen.
- The lack or inappropriateness of local and systemic antibodies to the virulence factors of the organism as well as the degree and specificity of the various cellular responses. These may be influenced by the severity of underlying disease, the degree of immunocompromisation and immunosenescence, and possible genetic polymorphisms in response genes.

From the bacterial side the amount and rate of production of the toxins together with the infectivity of the bacterium and perhaps its degree of sporulation will also influence disease severity. One of the reasons why ribotype 027 strains are thought to be hypervirulent is because of increased toxin production. These stains seem to over-produce toxins as a result of a deletion in their *tcdC* gene - the negative regulator of toxin transcription. Similar deletions are found in other strains not currently recognised as being hypervirulent.

The perception that it is only the elderly, hospitalised, antibiotic-treated patients who are susceptible has been challenged recently with more reports of community-acquired cases, disease in younger patients and in peripartum women. However, although such reports are increasing, the observations are not really new and the perception is probably due to an increasing number of cases and increased awareness. However, there is little doubt that the emergence of hypervirulent strains such as those belonging to ribotype 027, and perhaps 078, are almost certainly more aggressive pathogens causing disease of increasing severity.

Clinical spectrum of *Clostridium difficile* Infection (CDI) and the emergence of hypervirulent strains; antibiotic and non antibiotic treatment.

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The illness associated with *Clostridium difficile* (*C. difficile* infection, CDI) ranges from mild diarrhoea to lifethreatening colitis. Typical clinical features include diarrhoea, lower abdominal pain and systemic symptoms such as fever, anorexia, nausea and malaise. Fulminant colitis occurs in 1–3% of patients, and is characterized by severe toxicity with high fever and diffuse abdominal pain and distention. Pseudomembranous colitis represents an advanced stage of disease, and although considered ‘non-specific’, it is highly suggestive of *C. difficile* infection. Recurrence of diarrhoea occurs in 10–40% of patients.

It is proposed that a severe case of CDAD is characterized by any of the following: admission to an intensive care unit for treatment of CDAD or its complication; surgery (colectomy) for toxic megacolon, perforation or refractory colitis; death within 30 days after diagnosis if CDI is either the primary or a contributive cause. So far, a severity grading system of CDI based on the amount and consistency of stools is lacking.

The three most frequently recognised risk factors for severe CDI are age, peak leukocytosis and serum creatinine. However, definitive severity markers await confirmation in prospective studies that preferably include a weighting for patient co-morbidities. Until this is available, any of the following may indicate severe CDI: WCC >15, acutely rising serum creatinine (e.g. >50% increase above baseline), elevated serum lactate, temperature >38.5 °C, or evidence of severe colitis (abdominal signs, radiology).

Recent outbreaks of CDAD with increased severity, high relapse rates and significant mortality have been related to the emergence of a new, hypervirulent *C. difficile* strain in 2003 in Canada, the United States and Europe. The predominant strain is referred to as North American pulsed-field type 1 (NAP1), PCR ribotype 027, and group BI by restriction endonuclease analysis. Strain NAP1 contains an 18-base pair (bp) and a deletion at 117 of the *tcdC* gene. This strain has been associated with the in-vitro production of toxins A and B in quantities 16 and 23 times, respectively, greater than production by control strains. A recent worrying new development is outbreaks in Europe due to clindamycin resistant NAP1/027 strains (MIC >256 mg/l), which harbour the *ermB* gene. Clindamycin has been considered as a “protective” antibiotic for the development of CDAD due to NAP1/027, but resistance to this agent increases the risk of CDI in patients, and its use may be an important factor contributing to the persistence and spread of this strain.

The first step in treating a patient with documented or suspected CDI is to discontinue treatment with the offending antimicrobial, when possible. Metronidazole has long been the first-line agent in the treatment of CDAD, but a decreasing effectiveness of metronidazole in CDAD has been documented. Treatment algorithms have been proposed to treat mild cases of CDI with metronidazole (500 mg 3 times per day) and to treat serious ill patients with CDI with vancomycin (125-250 mg 6-hour). CDI caused by *C. difficile* ribotype 027 has been associated with a significantly higher metronidazole failure rate than cases due to other *C. difficile* types. Metronidazole failure may be due to low dosage and/or poor pharmacokinetics. There is evidence that inactivation of metronidazole occurs in the presence of gut contents, possibly due to metabolism by enterococci. Recently, the emergence of reduced susceptibility to metronidazole in UK *C. difficile* strains has been reported.

Definitive conclusions for the optimal treatment of CDI can only be obtained through randomized controlled trials that account for differences in case severity and other potential confounding factors including host response. Several new antibiotic and non-antibiotic alternatives have become available as alternatives for vancomycin and metronidazole, but there is currently no place for probiotic treatments.

Clostridia: clinical issues

Clostridial infections in immunocompromised hosts

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Immunocompromised hosts have a high incidence of risk factors associated with *Clostridium difficile*-associated colitis (CDAD) such as prolonged hospitalisation, antimicrobial use, immunosuppression and surgical procedures. Furthermore key steps in the host response to CDAD are modified in this patient group both by the underlying medical conditions or their treatment, in particular signal transduction pathways and cytokine responses to toxins produced by *C. difficile*. Diarrhoea is frequent posttransplantation and in HIV-positive individuals and CDAD is a common cause contributing to significant morbidity. CDAD is a significant cause of hospital-acquired infection in both groups. In solid organ and haematopoietic stem cell transplant recipients the cumulative incidence of CDAD can reach up to 15%. Risk factors both common to each and specific to each transplant group have been defined. While CMV disease in general has not been demonstrated to be a significant risk factor hypoalbuminemia is a risk factor in certain patient groups. For HIV-positive individuals in addition to traditional risk factors low CD4 T-cell counts are associated with increased incidence and successful treatment with highly active antiretroviral therapy has reduced CDAD incidence. Clinical presentations are similar in both transplant recipients and HIV-positive patients though fever may be less common in transplant recipients and a leukemoid reaction is relatively uncommon in immunocompromised individuals. The question of whether CDAD is more likely to be complicated in immunocompromised patients is confounded by bias in existing series although series of CDAD patients requiring colectomy contain significant numbers of immunocompromised hosts. Links between CDAD and graft dysfunction or graft-versus-host disease do not confirm whether associations represent cause or effect. CDAD is also associated with an increased risk of colonisation with vancomycin resistant enterococci. Diagnosis includes conventional microbiological approaches but sensitivities may be lower in immunocompromised patients and CT scanning of the abdomen may be required. In general therapy is similar to other patient groups but early colectomy should be considered in select patients and case series suggest intravenous immunoglobulin may be a consideration in patients with predefined baseline characteristics. A small number of reports exist of non-difficile related clostridial infections and these include blood stream infections, gas gangrene formation and toxin-mediated syndromes.

Emerging clostridial infections in the USA

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Over the past decade, *Clostridium* spp. possessing large clostridial toxins have emerged or re-emerged to impact the epidemiology of infections in the United States. In 1999 and 2000, six black-tar heroin users developed necrotizing fasciitis due to *Clostridium sordellii*, three of whom died. Like similar cases in Scotland, Ireland, and England caused by *Clostridium novyi*, infections resulted from contamination of the heroin that was injected subcutaneously. In 2001 a 23-year old man died of *C. sordellii* sepsis following a bone and cartilage allograft. Despite it being a rare cause of clinical infection overall, *C. sordellii* is among the most common contaminants of cadaveric tissue allografts. Animal studies suggest *Clostridium* spp. spores may lie dormant in skeletal muscle *in vivo*; there are reports of human *C. sordellii* infection, unrelated to allografts, which have occurred following blunt as well as penetrating trauma to skeletal muscle. In addition, *C. sordellii* can rarely cause toxic shock syndrome (TSS) in peripartum women. Between 2001 and 2008 there were seven reported cases of TSS in North America, including six fatal cases associated with medical abortion. In all but the one non-fatal case not associated with medical abortion, diagnosis was confirmed by amplification of *C. sordellii* genomic sequences from formalin fixed tissues, including amplification of sequences encoding lethal toxin (TcsL). The one non-fatal peripartum case during this period was caused by a TcsL-negative strain. Meanwhile, from 2000 through 2006, both rates and absolute numbers of U.S. hospitalizations associated with *Clostridium difficile* infection (CDI) have increased from two to three-fold. Death certificates indicating CDI as a cause of death increased nearly four-fold from 1999 to 2005. Recent studies indicate CDI-attributable mortality ranges from 5 to 16%, depending upon consideration of indirect, in addition to direct, mortality, the time period over which mortality is measured, and whether or not it is measured during an outbreak. This is increased over historic figures for CDI mortality ranging from 1-2%. Excess healthcare costs attributable to CDI total more than \$1 billion annually for the United States alone. Increased CDI incidence and mortality appears due to spread of an epidemic strain known variously as North American Pulsed-field type 1 (NAP1), restriction endonuclease analysis type "BI", or PCR ribotype 027. NAP1/BI/027 carries an extra toxin in addition to toxins A and B, known as binary toxin, and possesses polymorphisms in the toxin negative regulatory gene, *tcdC*. The latter may be in part or wholly responsible for toxin hyper-production with 16-fold increased toxin A production and 23-fold increased toxin B production documented *in vitro*. This strain, although historically uncommon, has become epidemic coincident with it becoming more resistant to fluoroquinolones. It has been identified in at least 38 states and throughout Canada. Previously low-risk populations in which CDI may be now more common include peripartum women, in whom disease may be life threatening, and persons living in the community including, but not limited to, otherwise healthy persons. There are several reports suggesting antimicrobial exposure, a ubiquitous factor for CDI in hospitalized patients, may be absent in a significant proportion of patients with community-associated CDI. Evidence is emerging that particular strains may migrate between animal and human populations with the food supply being one possible route for such transmission.

Clostridia in cancer therapy

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Cancer is a disease with a high incidence in the western world. The use of conventional treatment modalities results in ~50% cure of cancer patients, whereas failure to control the tumor locally and metastasis result in treatment failure in the other 50%. To increase treatment potential, substantial resources are currently being devoted to discovery and development of new anti-cancer therapies. Included amongst these new approaches is gene therapy, with a wide variety of proposed therapeutic genes. One of the most important problems in the development and use of gene therapy is the safe and specific delivery of genes to the tumor. A variety of viral vector delivery systems and a number of non-viral mechanisms have been developed and many different approaches have been conceived to produce more selective vector systems. When making choices regarding suitable vectors for gene therapy for cancer, it is important to recognize both the factors that distinguish a tumor from its surrounding normal tissue as well as the factors that limit successful therapy with available treatments. Tumor hypoxia is a good example. In that context, an alternative to viral delivery that has been proposed is the use of non-pathogenic anaerobic bacteria, predominantly *Clostridium* spp., as gene delivery vehicles.

The concept of this approach has been around for more than 50 years and is based on the unique physiology of solid tumors which is often characterized by regions of hypoxia and necrosis. Systemically injected spores from a variety of clostridial strains in tumor-bearing animals have been shown to localise and germinate only in hypoxic and necrotic regions of solid tumours. The application of clostridia as delivery vehicle has proven to be highly feasible and safe. Importantly, treatment of experimental tumours with genetically engineered clostridia has yielded significant therapeutic effects. This therapeutic efficacy is due not only to the fact that clostridia show a high selectivity for hypoxic tumour areas, but also because these hypoxic areas are considered one of the most important barriers to current cancer treatment options. The approach may thus act in a complementary way to current radiotherapy and chemotherapy treatments.

An overview of the current status of anaerobic bacteria use in cancer treatment will be provided.

Toll-like receptors and intestinal inflammation

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Toll-like receptors (TLRs), a family of molecules that recognize microbial products, are an essential component of the innate immune system. The microbial products recognized by TLRs are characterized as pathogen-associated molecular patterns (PAMPs), although they are produced by commensal organisms as well as pathogens. TLRs act as sensors of microbial invasion; activation of TLRs results in the generation of chemokines and cytokines to limit the extent of microbial invasion. The TLR family includes TLR4, which recognizes LPS, and TLR2, which recognizes bacterial lipoprotein/lipoteichoic acid. MyD88 is an adaptor molecule for all of the TLRs; mice lacking MyD88 are unable to respond to any of the TLR ligands.

Oral administration of dextran sodium sulfate (DSS) induces colitis in mice. Mice lacking TLR2, TLR4 or MyD88 all develop more severe colitis than wild type mice when exposed to orally administered DSS. The more severe colitis in the TLR2^{-/-}, TLR4^{-/-} and MyD88^{-/-} mice is associated with diminished colonic epithelial proliferation. Administration of DSS plus broad spectrum antibiotics to wild type mice results in DSS colitis of the same severity as was seen in TLR2^{-/-}, TLR4^{-/-} and MyD88^{-/-} mice. These findings suggest that signals from commensal bacterial through TLRs results in protection from DSS colitis through enhanced epithelial proliferation. In contrast to their crucial role in the regulation of epithelial proliferation in the face of DSS injury, TLRs appear to have no role in the regulation of epithelial proliferation in the healthy mouse. In the absence of injury, colonic architecture and epithelial proliferation are identical in TLR2^{-/-}, TLR4^{-/-}, MyD88^{-/-} and wild type mice. Subsequent studies demonstrated that cyclooxygenase-2 (COX-2) expression and the production of prostaglandin E₂ (PGE₂) were important downstream signaling events for TLR signaling in the DSS model. Administration of DSS to COX-2^{-/-} mice resulted in the same worsening of DSS colitis and the same diminished epithelial proliferation as was seen with administration of DSS to MyD88^{-/-} mice. Administration of a stable PGE₂ analog, dimethyl PGE₂, resulted in the rescue of DSS colitis in both the MyD88^{-/-} and the COX-2^{-/-} mice but had no effect on DSS colitis in wild type mice. Administration of DSS to wild type mice results in the migration COX-2 expressing mesenchymal stem cells from sites remote from the intestinal epithelium to a site immediately adjacent to the intestinal epithelium. In MyD88^{-/-} mice the administration of DSS does not induce the migration of these COX-2 expressing mesenchymal stem cells. These data suggest that in this model of colonic injury epithelial proliferation is preserved by the secretion of PGE₂ by COX-2 expressing mesenchymal stem cells which migrate in response to a TLR mediated signal. Subsequent studies demonstrated that TLR signaling, mediated through COX-2 expression and PGE₂ production, is a generalized protective mechanism for injured gastrointestinal epithelium. This mechanism has been demonstrated to be an important not only in DSS induced colitis but in radiation induced injury to the small intestinal epithelium and in acid ethanol induced injury in the stomach.

Clinical-epidemiological characterization of Clostridium difficile-Ribotype 053 - a new strain with high transmissibility

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BACKGROUND:

Hypervirulent strains of *Clostridium difficile* are causing hospital outbreaks in Europe.

This phenomenon may not only be due to PCR ribotype 027.

Hospital surveillance programs should therefore not only focus on this ribotype but also on other ribotypes.

METHODS:

Since February 2006 to Oct.07 a laboratory based surveillance program has been in place. All patients with diarrhea are obligatory screened for CDAD.

Case definitions according to the *Clostridium difficile* working group recommendations (ECDC) were used for case identification.

RESULTS:

514 patients fulfilled the definition criteria of a case of CDAD in the period of 19 months.

Of these 514 cases, 97 (19%) cases were selected cases because of the severe course of their CDAD episode, relapse of CDAD cases and because of having been related in time and/ or space with the severe or relapse cases.

There was no ribotype 027 found among these isolates.

The most prevalent ribotype found was ribotype 053 (49; 48%)

39 out of 49 ribotype 053 were investigated clinically-epidemiologically. 15 showed a severe course of CDAD (38%). 13 showed a fatal course directly or indirectly related to CDAD (case-fatality: 33%). Ribotype 053 showed not only a high case-fatality-ratio (0,3) but was also suspected to be highly transmissible. It was responsible at least for two clusters at the gastroenterology-department.

CONCLUSION:

Based on ribotyping one major strain - PCR-Ribotype 053 - was identified in a surveillance period of 19 months. Our findings indicate a high transmissibility and hypervirulence of this strain.

Characterization of *Clostridium difficile* ribotype 078 from human and animal origin

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Background: *Clostridium difficile* PCR-ribotype 078 is the predominant ribotype in cattle and seems to be an emerging type in human disease. To characterize human (n=43) and pig (n=8) PCR-ribotype 078 isolates we applied Multi Locus Variable tandem repeat Analysis (MLVA) and investigated the isolates for the presence of resistance gene (ermB) and genes encoding for toxins. Additionally, we sequenced the negative regulator (tcdC).

Methods: The presence of the toxin and ermB genes was established by conventional PCR. Sequencing of the tcdC gene was performed by cycle sequencing and analysis. Susceptibility to moxifloxacin, ciprofloxacin, clindamycin and erythromycin was investigated using E-test method.

Results: All strains tested positive for the toxin genes. The ermB gene was present in 14% of the strains. All characterized isolates contained two point mutations and a 39 bp deletion in the tcdC gene. Resistance for moxifloxacin, ciprofloxacin, clindamycin and erythromycin was found in 12%, 88%, 6% and 75% of the isolates respectively. MLVA revealed that, 97% of the tested human and pig isolates were genetically related. Two genetically highly related clusters were found containing both human and pig isolates.

Conclusion.

1. Toxin profiles of human and pig isolates were identical 2: Most of the clindamycin and erythromycin resistant isolates (66%) are ermB positive. 3: The point mutation introduces a stopcodon in the tcdC gene leading to a non-functional tcdC protein. 4. MLVA shows that almost all tested type 078 strains were genetically related and that 59% of human and pig isolates were even genetically identical.

Isolation of Clostridium difficile from domestic and wild avian species

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Clostridium difficile infection has been reported in various farm animals, such as pigs and calves. Our previous data are suggesting that situation in poultry farms is quite different to the other animal hosts in terms of higher isolation rates and higher diversity of genotypes found in chickens. The purpose of this study was to assess the carriage of *C. difficile* in different household and wild avian species to determine their potential as a reservoir of *C. difficile*.

Animals included in the study were: farm pheasants and partridges, quail, geese, turtledove, peacock, crow and six different backyard chicken populations. Isolates were cultured from collected fecal samples after enrichment in the selective medium and alcohol shock and were characterized by toxinotyping and ribotyping.

From domestic chickens from 0% to 50% of fecal samples yielded *C. difficile*. This is lower prevalence than observed previously for a large farm (71,4% to 95,8% of culture positive fecal samples). All known chicken isolates belonged to 12 different ribotypes. Two strains were nontoxigenic, while toxinogenic strains belonged to toxinotype 0 and IV (binary toxin positive). *C. difficile* was also isolated from partridges (40%), geese (33,3%) and crow. Partridge isolates were toxinotype XIb (binary toxin positive) and XII. Geese and crow isolates were toxinotype 0. *C. difficile* isolates from avian species has shown similarities in PCR ribotype with isolates from pigs, calves and humans.

These results show that domestic and wild avian species seems to be a suitable host for *Clostridium difficile* colonization.

Clinical significance of clostridial bacteremia: a retrospective three-year analysis in a French University Hospital Center

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Background

Anaerobic bacteremia has recently reemerged with *Clostridium spp.* being the most common isolated organisms after *Bacteroides* species. However, the clinical significance of clostridial bacteremia is still controversial.

Objective and methods

To evaluate the clinical impact of clostridial bacteremic episodes, all *Clostridium* bacteremia cases that occurred in patients hospitalized at our center during 2004-2006 were retrospectively reviewed.

Results

Clostridial bacteremia was observed in 41 patients (mean age: 61.7 years) of whom 11 had severe sepsis and 4 had septic shock. The most common *Clostridium* species was *C. perfringens* (34.1%) followed by *C. ramosum* (21.9%). Bacteremic episodes were monomicrobial in 39% of the cases and occurred predominantly in patients with underlying malignancy (51%) and for whom a digestive portal of entry was suspected (58%). A clinical relevance rate of 48.8% was found when clinical significance was considered if at least one blood culture bottle was positive for *Clostridium spp.* while a compatible source of infection and/or signs of severe sepsis or shock were observed. When clinical significance analysis was restricted to cases in which at least two blood samples were cultured (n=28), a rate of 42.8% was found using Benjamin's criteria (clinical significance if ≥2 blood culture bottles positive for *Clostridium spp.* and presence of a compatible source of infection and/or signs of severe sepsis or shock).

Conclusion

Clostridium bacteremia may have a major clinical impact which warrants adapted antibiotic therapy. The systematic use of at least two anaerobic blood cultures would be preferable to ensure proper clinical interpretation.

Risk factors for Clostridium difficile infections in an endemic setting in The Netherlands

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Background.

After emerging outbreaks of *Clostridium Difficile* Infection (CDI) in Canada and the United States, outbreaks of CDAD were also reported in The Netherlands. Few studies are available on risk factors for CDI in endemic situations.

Methods.

From July 2006 through December 2007, all received diarrhoeal stool samples from clinical patients at the LUMC were tested for CDI, regardless the physicians' request. A study was initiated to investigate incidence of CDI and to identify risk factors for the disease in the endemic situation. We compared demographic and clinical data among cases (n=43) and both controls without diarrhoea (n=43) and controls with diarrhoea but with a toxin test negative for *C. difficile* (n=30).

Results.

The CDI incidence was 2.1 per 10.000 inpatient-days. Compared to both control groups, significant risk factors in multivariate analysis for CDI were age above 65 years, hospitalization and use of antibiotics, notably 2nd generation cephalosporins, in the 3 months prior to CDI. Control patients with diarrhoea, tested negative for CDI, significantly more often used 3rd generation cephalosporins than control patients without diarrhoea. The total number of antibiotics and received Defined Daily Doses (DDD?s) were also significantly higher in this control group.

Conclusions.

The results of this study suggest that previous hospitalization, older age and the use of 2nd generation cephalosporins are independent risk factors for CDI in this endemic situation. The total number of antibiotics and received DDD?s are risk factors for the development of diarrhoea, not specifically CDI.