

# THE EMERGENCE OF *CLOSTRIDIUM DIFFICILE* PCR RIBOTYPE 027 IN DENMARK – A POSSIBLE LINK WITH THE INCREASED CONSUMPTION OF FLUOROQUINOLONES AND CEPHALOSPORINS?

L Søes (LMS@ssi.dk)<sup>1</sup>, K Mølbak<sup>2</sup>, S Strøbæk<sup>3</sup>, K Truberg Jensen<sup>4</sup>, M Torpdahl<sup>1</sup>, S Persson<sup>1</sup>, M Kemp<sup>1</sup>, K E Olsen<sup>1</sup>

1. Department of Bacteriology, Mycology and Parasitology, Statens Serum Institut, Copenhagen, Denmark

2. Department of Epidemiology, Statens Serum Institut, Copenhagen, Denmark

3. Department of Infection Control, Sydvestjysk Hospital, Esbjerg, Denmark

4. Department of Clinical Microbiology, Sydvestjysk Hospital, Esbjerg, Denmark

Increasing rates of *Clostridium difficile* infection (CDI) with an unusual, severe course have been reported in several countries; this rise has partly been ascribed to the emergence of a virulent strain, *C. difficile* PCR ribotype 027 (CD027). An intriguing question is whether this could be related to increasing consumption of broadspectrum antibiotics. From 1997 to 2007, the number of hospital discharges in Denmark with the diagnosis enterocolitis caused by *C. difficile* increased from eight to 23 per 100,000 hospital discharges. This increase was proportional to a concomitant rise in the consumption of fluoroquinolones and cephalosporins. The first outbreak of CD027 in Denmark occurred from October 2006 to August 2007 and included 13 patients, most of them elderly, admitted to three hospitals in the same region. Most of the patients had overlapping periods of admission. All patients had been treated with broadspectrum antibiotics, in particular cephalosporins and fluoroquinolones, prior to positive culture of CD027. Thirty days after confirmation of diagnosis, three of the 13 patients had died. Taken together, the data support the hypothesis that the increasing use of certain broadspectrum antibiotics may be related to a possible increase of *C. difficile* infection, and show that the specific contribution by CD027 in its emergence needs to be determined.

### Introduction

Infection with toxin-producing strains of *Clostridium difficile* is a common cause of diarrhoea and varies from mild to severe cases of diarrhoea. Cases are frequently antibiotic-associated and occur mostly in hospitals. Pseudomembranous colitis in already impaired patients e.g. with an underlying condition is a serious manifestation of *C. difficile* infection (CDI) and can result in death.

Reports from North America, Europe and Japan have drawn attention to a recently discovered strain of *C. difficile* that is characterised as PCR ribotype 027, toxinotype III (CD027) [1-4]. This strain has an increased pathogenic capacity, possibly a higher infectious potential and a particular resistance profile. The increased pathogenicity is thought to be associated with an enhanced production of toxin A and toxin B caused by mutations

in a regulatory gene, but the fact that this strain in addition produces a binary toxin CDT may also contribute to increased pathogenicity. This strain has caused severe outbreaks of CDI in hospital environments, but has also been described as the cause of outbreaks and sporadic cases outside hospitals [2-4].

The aims of the present report are to summarise national hospital data with a discharge diagnosis of CDI and to describe the first outbreak of CD027 in Denmark.

### Methods

Because of the international emergence of CD027 and the subsequent recommendations from ECDC [5], we obtained hospital discharge data on CDI in Denmark from 1997 to 2007 and conducted a retrospective characterisation of *C. difficile* isolates from November 2006 to March 2007. In addition, Statens Serum Institut (SSI) asked Danish departments of clinical microbiology to continuously report *C. difficile* findings and to forward isolates for typing on suspicion of an outbreak or severe disease.

The hospital discharge data were obtained from the statistics of the Danish National Board of Health (<http://sundhedsdata.sst.dk>). Specifically, we obtained the annual aggregated number of discharges with the ICD10 diagnosis code DA04.7 ("enterocolitis caused by *C. difficile*", i.e. enterocolitis independent of PCR ribotype) as well as the annual number of all discharges from somatic hospitals, i.e. hospitals treating only somatic and not psychiatric diseases. Data about consumption of fluoroquinolones and cephalosporins were obtained from The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) [6].

Isolates of *C. difficile* were characterised by PCR ribotyping, toxin gene profiles, and deletion studies undertaken by the National Reference Laboratory for Enteropathogenic Bacteria at SSI.

Stool samples were cultured on cycloserine cefoxitin fructose agar (CCFA) (SSI Diagnostica, Hillerød, Denmark) in an atmosphere composed of 86% N<sub>2</sub>, 7% H<sub>2</sub> and 7% CO<sub>2</sub> at 37°C for 48 hours.

Colonies with typical morphology and distinctive odour were identified. The colonies were analysed by 5-plex PCR directed towards *tcdA*, *tcdB*, *cdtA*, *cdtB* and 16S rDNA and by sequencing of the 5'-end of *tcdC* in order to search for premature stop codons and internal deletions [7]. PCR ribotyping was performed according to Bidet *et al.* [8].

## Results

### Hospital discharges of CDI in Denmark

The aggregated number of discharges of enterocolitis caused by *C. difficile* increased from 86 (eight per 100,000 discharges) in 1997 to 282 (23 per 100,000 discharges) in 2007. In the same period, the consumption of fluoroquinolones and cephalosporins used in primary healthcare and hospitals taken together, increased

from 384 to 1,162 kg and from 626 to 2,285 kg active component per annum, respectively (see Figure 1) [6].

### Detection of CD027 in Denmark

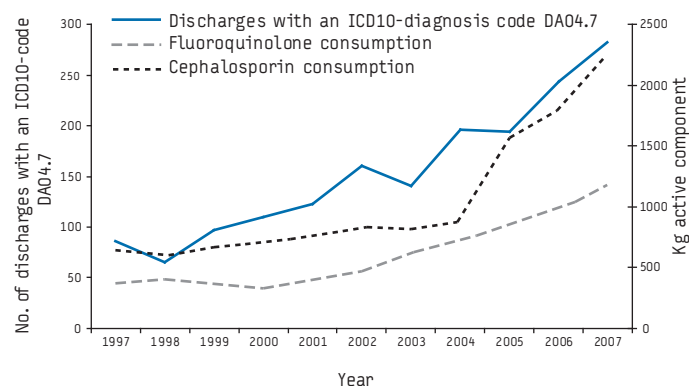
In the retrospective survey, isolates obtained between November 2006 and March 2007 were characterised; eight CD027 cases were found (Figure 2). The isolates came from eight hospitalised patients from the Region of Southern Denmark (the former Ribe County). Seven of the patients had been admitted to the same small hospital A, while the last case was a patient in another hospital in the same local area. Prompted by this cluster, active surveillance for CD027 was established in the area, and an additional 22 isolates of *C. difficile* were received between June and August 2007, of which five were CD027 (see Figure 2).

Thus, a total of 13 patients with CD027 were identified. Mean age was 79 years (age range 64 to 96 years), and 10 cases were women. The patients were admitted to hospital in the period October 2006 to July 2007. Nine of the patients were admitted to the same medical ward at the small hospital A, which consisted of only the one ward and a surgical day clinic. Most of these patients had overlapping periods of admission. The CD027-positive stool sample from one of these patients was requested by the general practitioner 13 days after the patient's discharge from hospital. The other eight were obtained during admission. The remaining four patients were admitted to three different medical departments at the larger hospital B. Two of these patients had overlapping periods of admission at the same ward. One of these four patients was moved to another medical ward at another small hospital C (see Figure 3).

The isolates were all PCR ribotype 027, carried the binary toxin gene, had an 18 bp deletion in the regulatory gene *tcdC*, and a 1 bp deletion at position 117 of *tcdC*. They were all resistant to fluoroquinolones (including moxifloxacin), but susceptible to erythromycin and clindamycin. Interestingly, at the same time and in the same geographical area, but unrelated to the outbreak

FIGURE 1

Annual number of hospital discharges with enterocolitis caused by *Clostridium difficile* (ICD10 diagnosis code DA04.7) and annual consumption of fluoroquinolones and cephalosporins for human use, Denmark, 1997-2007



Source: [6]

FIGURE 2

Number of patients with *Clostridium difficile* infection caused by CD027, Denmark, October 2006-August 2007 (n=13)

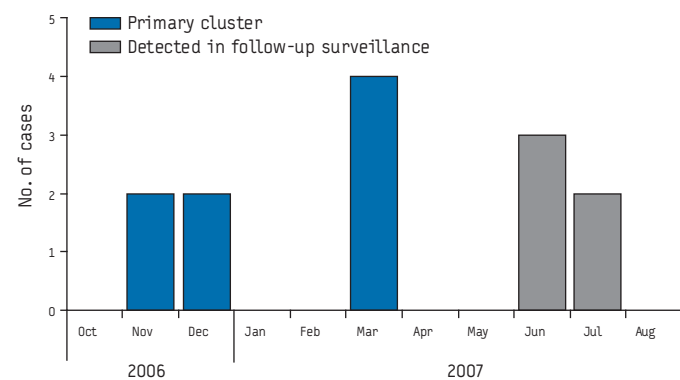
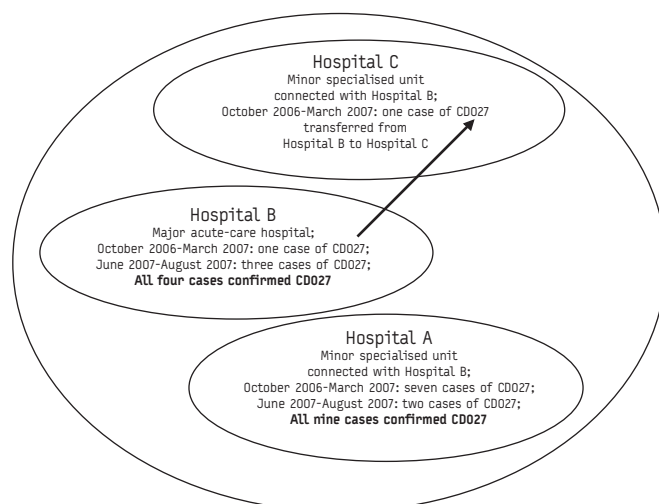


FIGURE 3

Distribution of cases of CD027 in the three hospitals, Denmark, October 2006-August 2007



another isolate was found that also carried the binary toxin gene, had the 18 bp and the 1 bp deletion at position 117 in the regulatory gene *tcdC*, but was not PCR ribotype O27. In contrast to the CD027 strains it was sensitive to moxifloxacin.

As this cluster of 13 cases was detected in a setting with ample possibilities of transmission and at the time represented the only detection of CD027 in Denmark, it is reasonable to assume that an outbreak with CD027 occurred during this period. Multilocus variable-number tandem-repeat analysis (MLVA) or restriction endonuclease analysis (REA) [9,10] will be performed in order to elucidate the connection between the isolates.

All of the 13 patients were treated with broadspectrum antibiotics prior to positive culture of CD027. Eleven patients received cephalosporins and nine fluoroquinolones; seven received both cephalosporins and fluoroquinolones, either simultaneously or consecutively. Thirty days after confirmation of diagnosis, three of the 13 patients had died. It is unknown if the deaths were directly attributable to *C. difficile*.

## Discussion

It is not known with certainty why the number of patients discharged after an episode of enterocolitis caused by *C. difficile* is increasing. However, it is certain that the patients with a discharge diagnosis of ICD10 code DA04.7 only comprise a modest fraction of the true number of cases. In 2007, 1,342 culture-confirmed cases of *C. difficile* infections were reported to the national surveillance system in Denmark (25 per 100,000 population). Surveillance was established in 2007. Data before this is therefore not available. Although increased diagnostic activity and awareness may play a role, it is also likely that changes in the strains' pathogenicity are important contributing factors to the emergence of CDI. This includes the appearance of CD027 and possibly other hypervirulent strains. Several factors may be of importance to understand the emergence of *C. difficile* and in particular of CD027. The CD027 strain is resistant to the newer fluoroquinolones, including moxifloxacin, and it has been suggested that this may be the main reason for its wide dissemination [2,3]. This hypothesis is supported by the almost parallel increase in CDI discharge diagnoses and the consumption of fluoroquinolones as illustrated in Figure 1. However it should be emphasised that resistance to moxifloxacin and several other fluoroquinolones is also seen in other *C. difficile* PCR ribotypes [11,12]. Furthermore, increased use of other broadspectrum antibiotics including cephalosporins may also be related to the emergence of *C. difficile* since the same almost parallel increase is observed in CDI discharge diagnoses and consumption of cephalosporins (Figure 1).

However, these possible relations should be interpreted with caution. Other circumstances may also be of considerable importance, such as the increasing challenges in the area of hospital hygiene. For example, increased virulence of *C. difficile* resulting in pronounced diarrhoeal symptoms may have promoted spread and cross-infection within healthcare institutions, possibly because of dissemination of spores by incontinent patients [3]. The emergence of *C. difficile* and CD027 in particular is likely to be a result of environmental as well as person-to-person transmission in healthcare facilities rather than solely a result of increased antibiotic pressure. Finally, demographic changes such as an age distribution with an increasing proportion of elderly people and

changes in the patterns of hospitalisation towards increased "turn-over" of patients may also contribute.

The recognition of the outbreak of CD027 in this particular geographical area of Denmark may not be an isolated observation. The initial cluster was detected in a convenience sample of stool specimens from diarrhoeal patients as part of a project including molecular characterisation of *C. difficile* isolates. Hence, it is conceivable that the cases discovered only represent the tip of the iceberg. On a voluntary basis, strains from all different geographical areas of Denmark are now being submitted for surveillance to the National Reference Laboratory for Enteropathogenic Bacteria to identify CD027.

Although we cannot conclude a cause-and-effect relation between the increase in fluoroquinolone and cephalosporin consumption and the increase in CDI discharge diagnoses, we consider it important to present these data to stimulate additional research. Studies are needed to determine the burden of disease associated with CD027 and other hypervirulent *C. difficile* strains, while integrated public health and microbiological surveillance should be established to determine trends, detect clusters in healthcare institutions, and facilitate more focused infection control. To prevent spread, it is essential to focus on hospital hygiene and promote prudent antibiotic policies, including the limitation of unnecessary use of broadspectrum antibiotics, including fluoroquinolones and cephalosporins.

## Acknowledgements

We wish to thank J. Nevermann Jensen for extensive technical assistance.

## References

1. McDonald LC, Killgore GE, Thompson A, Owens RC, Kazakova SV, Sambol SP, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med*. 2005;353(23):2433-41.
2. Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med*. 2005;353(23):2442-9.
3. Waryn M, Pepin J, Fang A, Killgore G, Thompson A, Brazier J, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet*. 2005;366(9491):1079-84.
4. Kuijper EJ, Barbut F, Brazier JS, Kleinkauf N, Eckmanns T, Lambert ML, et al. Update of *Clostridium difficile*-associated disease due to PCR ribotype 027 in Europe, 2008. *EuroSurveill* 2008;13(31):pii=18942. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18942>
5. Kuijper EJ, Tüll P. Emergence of *Clostridium difficile*-associated disease in Canada, the United States of America and Europe. Background document prepared on behalf of the European Study Group for *Clostridium difficile* (ESGCD) and European Centre for Disease Prevention and Control (ECDC). 2006 March 3. Available from: [http://www.ecdc.europa.eu/documents/pdf/CL\\_diff\\_v2.pdf](http://www.ecdc.europa.eu/documents/pdf/CL_diff_v2.pdf)
6. DANMAP 2007. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. DANMAP – The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. 2008 September. ISSN 1600-2032. Available from: <http://www.danmap.org>
7. Persson S, Torpdahl M, Olsen KE. New multiplex PCR method for the detection of *Clostridium difficile* toxin A (*tcdA*) and toxin B (*tcdB*) and the binary toxin (*cdtA/cdtB*) genes applied to a Danish strain collection. *Clin Microbiol Infect*. 2008;14(11):1057-64.
8. Bidet P, Barbut F, LaLande V, Burghoffer B, Petit JC. Development of a new PCR-ribotyping method for *Clostridium difficile* based on ribosomal RNA gene sequencing. *FEMS Microbiol Lett*. 1999;175(2):261-66.

9. Fawley WN, Freeman J, Smith C, Harmanus C, van den Berg RJ, Kuijper EJ et al. Use of Highly Discriminatory Fingerprinting to Analyze Clusters of *Clostridium difficile* Infection Cases Due to Epidemic Ribotype 027 Strains. *J Clin Microbiol*. 2008;46(3):954-60.
10. Killgore G, Thompson A, Johnson S, Brazier J, Kuijper E, Pepin J, et al. Comparison of Seven Techniques for Typing International Epidemic Strains of *Clostridium difficile*: Restriction Endonuclease Analysis, Pulsed-Field Gel Electrophoresis, PCR-Ribotyping, Multilocus Sequence Typing, Multilocus Variable-Number Tandem-Repeat Analysis, Amplified Fragment Length Polymorphism, and Surface Layer Protein A Gene Sequence Typing. *J Clin Microbiol*. 2008;46(2):431-7.
11. Wilcox MH, Fawley W, Freeman J, Brayson J. In vitro activity of new generation fluoroquinolones against genotypically distinct and indistinguishable *Clostridium difficile* isolates. *J Antimicrob Chemother*. 2000;46(4):551-6.
12. Alonso R, Peláez T, González-Abad MJ, Alcalá L, Muñoz P, Rodríguez-Crêixems M et al. In vitro activity of new quinolones against *Clostridium difficile*. *J Antimicrob Chemother*. 2001;47(2):195-7.

This article was published on 16 April 2009.

Citation style for this article: Sørensen L, Mølbak K, Strøbæk S, Truberg Jensen K, Torpdahl M, Persson S, Kemp M, Olsen KE. The emergence of *Clostridium difficile* PCR ribotype 027 in Denmark – a possible link with the increased consumption of fluoroquinolones and cephalosporins?. *Euro Surveill*. 2009;14(15);pii=19176. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19176>