

OUTBREAK OF *CLOSTRIDIUM DIFFICILE* 027 IN NORTH ZEALAND, DENMARK, 2008-2009

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We report an outbreak of *Clostridium difficile* PCR ribotype 027 in Denmark. The outbreak includes to date 73 cases from the area north of Copenhagen, but there may be related cases elsewhere in Zealand. Most infections are healthcare-associated and in patients who previously received antibiotic treatment. The strain is resistant to moxifloxacin, erythromycin, and clindamycin, and carries genes for toxin A, toxin B, and for the binary toxin. The antimicrobial pattern differs from that of the strain involved in a small cluster in Denmark in 2006-2007. Because of this outbreak, hygienic measures in the involved hospitals have been reinforced. Nationwide, microbiological laboratories were alerted to the outbreak and encouraged to send isolates for toxin profiling and PCR ribotyping.

Introduction

Clostridium difficile infection is the leading cause of nosocomial diarrhoea in industrialised countries. A specific subtype, *C. difficile* PCR ribotype 027 has been associated with more severe disease and caused outbreaks in North America and Europe [1-3]. The increased virulence is assumed to be associated with higher amounts of toxin production [2-4].

A cluster of 13 cases of *C. difficile* 027 occurring in southern Denmark between November 2006 and July 2007 was identified as part of a retrospective survey in 2007. The outbreak strain carried the binary toxin genes and was resistant to fluoroquinolones, and susceptible to erythromycin and clindamycin [5]. Since then, Danish departments of clinical microbiology were asked to report *C. difficile* findings and to forward selected isolates for toxin profiling and PCR ribotyping to the National Reference Laboratory at Statens Serum Institut, in particular whenever a severe disease or an outbreak was suspected.

A possible outbreak of infections caused by a strain of *C. difficile* resistant to moxifloxacin, erythromycin, and clindamycin, as determined by the Oxoid disk diffusion method, was recognised in January 2009 by the Department of Clinical Microbiology in Hillerød Hospital. This strain was confirmed by Statens Serum Institut as PCR ribotype 027. The Department of Clinical Microbiology undertakes diagnostics for the North Zealand area (i.e. north of Copenhagen), including four hospitals and one rehabilitation clinic. We conducted an investigation to assess whether there was

an outbreak and to determine if the infections were healthcare-associated.

Methods

We used descriptive epidemiology to characterise the outbreak. Data on cultures and antibiotic resistance profile were collected at the Department of Clinical Microbiology, while toxin profiles and PCR ribotyping were obtained from the Statens Serum Institut. Additional information on symptoms, antibiotic treatment, and dates of hospital stay was collected from the electronic health records for the 60 days preceding the isolation of *C. difficile*.

For the purpose of the investigation, the following operational case definitions were adopted:

- A possible case was defined as a patient with a positive culture of *C. difficile* resistant to moxifloxacin, erythromycin, clindamycin;
- A probable case was defined as a patient with positive *C. difficile* culture and the presence of genes for toxin A, toxin B, and binary toxin;
- A confirmed case was defined by positive culture of *C. difficile* PCR ribotype 027;
- A relapse was defined as the occurrence of a second episode of *C. difficile* isolation (possible, probable, or confirmed as above) within 60 days from the first episode.

We considered the date of diagnosis as the date on the request form of the first positive stool sample in the Department of Clinical Microbiology. All stool samples from hospitalised patients were routinely tested for *C. difficile*. Toxin testing was performed on all cultures of *C. difficile* and on faeces in clinically obvious cases.

C. difficile isolates were characterised by toxin analysis (determining the genes for toxin A, toxin B and the binary toxin) and PCR ribotyping. On 48 isolates we also performed DNA sequencing searching for unique mutations in the regulating toxin gene tcdC (18 bp deletion and 1 bp deletion at position 117 of tcdC).

Current situation

From week 29, 2008 to week 15, 2009, a total of 73 cases (11 possible, eight probable and 54 confirmed cases) were recorded. As of week 15, 2009, all but one possible case have been confirmed

as O27. All 48 isolates DNA sequenced carried the mutations in the regulating toxin gene *tcdC* (the 18 bp deletion and the 1 bp deletion at position 117 of *tcdC*).

Three of the four North Zealand hospitals mentioned above and the rehabilitation clinic were involved.

We undertook a descriptive study of the first 59 consecutive cases since July 2008. A total of 32 of 59 cases were female and the median age was 81 years (interquartile range 73-87 years). A total of 53 of 59 cases were diagnosed among hospitalised patients; the mean time from admission to diagnosis was 9.5 days (range 0-72 days). Two other cases were sampled while in the emergency room; both had been previously hospitalised. The other four cases were diagnosed during an outpatient visit, or in a general practice. However, they had all had contact with a hospital in the 60 days prior to the diagnosis.

Forty-two of 59 cases were diagnosed more than two days after admission and therefore fulfil the criteria of healthcare-associated cases [4].

Up to week 10, 2009, we recorded 13 deaths occurring after the *C. difficile* diagnosis. Medical history was reviewed by two physicians and in eight cases, six of which had underlying conditions, *C. difficile* might have been a contributory cause of death.

Up to week 10, 2009, nine relapses were observed within 60 days after the first diagnosis. The median time between two infections was 31 days (range 23-50 days). Overall 68 episodes occurred (59 first infections and nine relapses). Diarrhoea with no systemic symptoms was reported in the medical records in 36 of them. Pseudomembranous colitis was reported in 20 episodes, toxic megacolon in two, and clinical sepsis in eight. In two of 68 episodes, symptoms were not described.

According to their hospital medical records, 55 of 59 cases had received antibiotics in the 60 days prior to the diagnosis, and 49 of the 59 cases had received two or more antibiotics.

The most commonly used antibiotics were: cephalosporins in 41, penicillins in 27, fluoroquinolones, mainly ciprofloxacin, in 25, and metronidazole in 20 of 59 cases.

To date (mid April), *C. difficile* O27 has been identified in other hospitals in Zealand, especially in other parts of the Copenhagen region. More specifically, from week 42, 2008 to week 15, 2009, a total of 243 isolates, including those from our investigation, were PCR ribotyped as O27 (128 in 2008 and 115 in 2009) by the National Reference Laboratory at Statens Serum Institut. Besides a possible presence of the strain in the community, the common practice of transferring patients between hospitals of the region might have contributed to the spreading.

Control measures

During the outbreak, hospitals' control measures were reinforced by extensive communication of the outbreak to the hospitals and by implementing the evidence-based strategy for *C. difficile* outbreaks [6], emphasising the need for good hand hygiene, isolation of patients, revision of environmental cleaning procedures, and collecting and storing faecal samples from cases for typing and possibly other analyses.

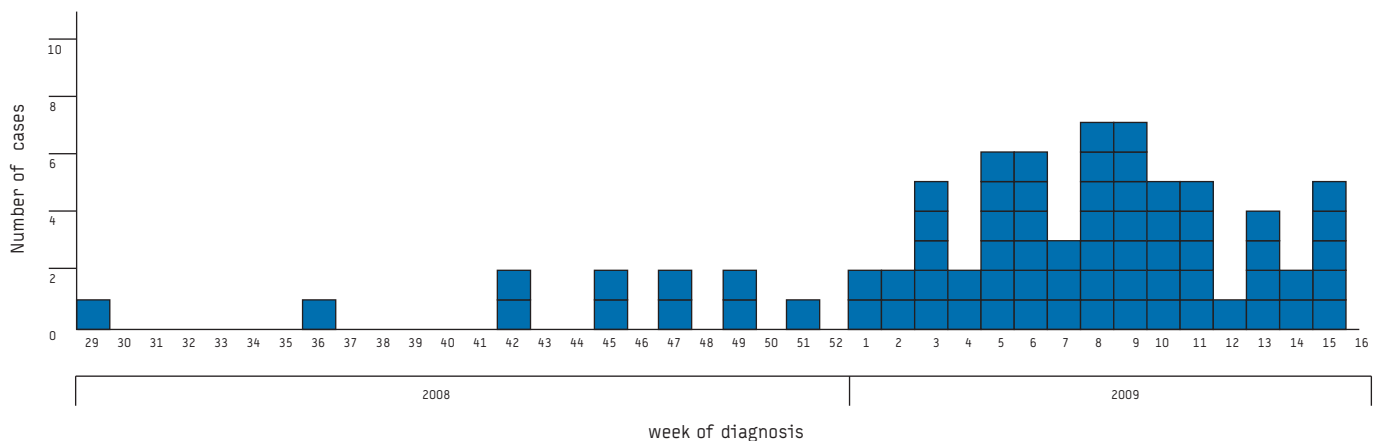
Because of the outbreak, the Danish National Board of Health decided to intensify the monitoring of *C. difficile* O27. All clinical microbiology departments, infection control organisations, and clinical departments in the country were advised to pay increased attention to possible cases of nosocomial diarrhoea, especially after antibiotic treatment. The National Board of Health also stressed that clinical microbiology departments are required to submit moxifloxacin-resistant isolates, isolates from cases with severe manifestations, and isolates collected during suspected outbreaks.

Discussion

We present preliminary data of the largest outbreak of *C. difficile* O27 recognised in Denmark. Most infections were healthcare associated, and almost all patients were treated with antibiotics

FIGURE

Distribution of confirmed (n=54), probable (n=8) and possible (n=11) *C. difficile* O27 cases, Denmark, week 29, 2008–week 16, 2009 (n=73)



Results are preliminary since PCR ribotyping of the isolates from the 19 probable/possible cases is pending.

in the two months prior to the *C. difficile* O27 isolation, foremost with penicillins, cephalosporins and fluoroquinolones. The present outbreak may be a part of the cases that have been observed in the Copenhagen region in an overlapping time period, and may represent an emergence of CDAD O27 in the capital region of Denmark. Based on resistance profile, this strain is different from the one described in Jutland in 2006-2007. This indicates the possibility of existence of more than one clone of *C. difficile* O27, with epidemic potential in Denmark. MLVA typing will help in disentangling these relations [7].

The outbreak has prompted increased attention to hospital hygiene, a coordinated response from regional and national authorities concerning surveillance and control, and regular communications between different microbiological laboratories in Zealand.

The number of cases in the last five weeks has levelled out as compared to previous weeks, which may indicate that the measures have taken effect. However, further monitoring is needed, as is continued vigilance regarding hygienic measures.

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