Clostridium difficile Ribotype 027, Toxinotype III, the Netherlands

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Outbreaks due to Clostridium difficile polymerase chain reaction (PCR) ribotype 027, toxinotype III, were detected in 7 hospitals in the Netherlands from April 2005 to February 2006. One hospital experienced at the same time a second outbreak due to a toxin A–negative C. difficile PCR ribotype 017 toxinotype VIII strain. The outbreaks are difficult to control.

Since March 2003, outbreaks of severe cases of Clostridium difficile–associated disease (CDAD) were reported in hospitals in Montreal and Quebec (1,2). Increased virulence was suspected, since the proportion of patients with CDAD who died within 30 days after diagnosis rose from 4.7% in 1991–1992 to 13.8% in 2003 (1). In addition, the Centers for Disease Control and Prevention reported a growing threat of CDAD in US hospitals and found the strain to be associated with high illness and death rates during hospital outbreaks in 11 states (3). The increased virulence was considered to be associated with the production of a binary toxin and an increased production of toxins A and B (4). Further characterization of this strain showed that it belonged to toxinotype III, pulsed-field gel electrophoresis (PFGE) type NAP1, restriction endonuclease analysis group BI, and polymerase chain reaction (PCR) ribotype 027 (2,3). Toxinotyping involves detecting polymorphisms in the toxin A and B and surrounding regulatory genes, an area of the genome known collectively as the pathogenicity locus or PaLoc (5). By toxinotyping, 24 different types can be recognized, whereas the library of PCR ribotypes comprises 116 distinct types of C. difficile identified on the basis of differences in amplification profiles generated (6). The PCR ribotype 027, toxinotype III, strain is resistant to ciprofloxacin and the newer generation of fluoroquinolones, such as gatifloxacin, levofloxacin, and moxifloxacin (3). Exposure of patients to fluoroquinolones and cephalosporins is recognized as a risk factor for CDAD caused by 027 (2,3). Increasing use of fluoroquinolones in US healthcare facilities may have provided a selective advantage for this epidemic strain and promoted its widespread emergence.

The Outbreaks

In July 2005, the medical microbiologic laboratory at the Leiden University Medical Center was requested to type C. difficile strains from an outbreak in a hospital (hospital l) in Harderwijk (Figure, Table). The incidence of CDAD in the hospital had increased from 4 per 10,000 patient admissions in 2004 to 83 per 10,000 admissions from April through July 2005. Cultured isolates were subsequently identified as toxinotype III and PCR ribotype 027 (7). The strain also had the binary toxin genes and contained an 18-bp deletion in a toxin regulator gene (tcdC). As determined by E test (AB Biodisk, Solna Sweden), the isolates were resistant to erythromycin (MIC >256 mg/L) and ciprofloxacin (MIC >32 mg/L) and susceptible to clindamycin (MIC 2 mg/L) and metronidazole (MIC 0.19 mg/mL). Measures taken by the hospital included isolating all patients with diarrhea until 2 tests were negative for C. difficile toxin, cohorting all C. difficile–infected patients on a separate ward, banning all fluoroquinolone use, and limiting use of cephalosporins and clindamycin.

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case-control study is being performed in the hospital to
determine risk factors for acquiring this strain, and a fol-
low-up study will determine the rate of complications and
relapses. As of January 2006, the situation appears to be
under control since the number of patients per month with
positive test results has decreased. All 9 CDAD cases from
September 2005 to January 2006 were caused by non-027
ribotypes. Therefore, cohort isolation and the limitation on
antimicrobial agents have been stopped.

A second epidemic occurred in another hospital 30 km
from the first hospital (hospital 2, Amersfoort) and was
probably related to the outbreak in hospital 1 through a
transferred patient with CDAD. Isolates obtained from
patients were indistinguishable from the Harderwijk iso-
lates. After the index patient was transferred, the incidence
of CDAD, which had been 2–3 cases per month for the last
2 years, rose to an average of 15 cases per month during
May, June, and July. From August to December, the num-
ber of CDAD patients per month was 7, 8, 14, and 10,
respectively. Of the 85 CDAD patients found through
December 2005, 19 (22%) patients died, and 16 (19%) had
relapses. Of 50 strains characterized at the reference lab-
atory, 15 belonged to PCR ribotype 027, and 14 belonged
to PCR ribotype 017, toxino type VIII. The 017 strain had
a deletion of the toxin A gene, did not contain genes for
binary toxin production, and had a normal \( tcdC \) gene.

In response to the outbreaks in the Netherlands, the
Centre for Infectious Disease Control at the National
Institute for Public Health and the Environment in
Bilthoven organized a meeting with experts in the fields of
microbiology, infectious diseases, infection control, and
epidemiology. The team agreed to combine parts of exist-
ing national hospital guidelines relevant for infection con-
trol of CDAD and to use national and international
experience in drawing up specific CDAD guidelines for
infection control and treatment separate for hospitals and
nursing homes. Diagnostic facilities were increased and
made accessible for all microbiology laboratories in the
Netherlands. Relevant professionals were informed
through different communication channels, including vari-
ous scientific societies (7). Plans were made to register and
monitor new outbreaks. Laboratories were encouraged to
send patient isolates or fecal samples for typing to the ref-

erence laboratory in Leiden when an outbreak was suspect-
ed on the basis of an increase in monthly incidence or a
rapid spread of clinically suspected cases.

Subsequently, 3 hospitals in the western part of the
country (hospitals 7–9) also reported an increase in inci-
dence of severe CDAD. In 2005, the public health labora-
tory serving these 3 hospitals diagnosed CDAD in 163
patients. Of 21 strains sent to the reference laboratory, 18
were identified as PCR ribotype 027, toxino type III
(Table). Retrospectively, an increase of CDAD was first
evident in July 2004 for hospital 7 and in 2002 for hospi-
tal 9. The public health laboratory diagnosed CDAD in 120
patients in 2004, in 58 in 2003, and in 47 in 2002. No
strains or fecal samples before 2005 were available for typ-
ing. A nursing home in the same region was also found to
have patients with CDAD due to PCR ribotype 027, with
evidence of spread within the facility. No epidemiologic
relationship could be established between this region ad
that of the first 2 outbreaks.

Two hospitals in the center of the Netherlands (hospi-
tals 3 and 4) did not notice an increase in the incidence of
patients with CDAD but submitted strains to the reference
laboratory for typing. Type 027 was found in 6 (35%) of 17
isolates and 1 (25%) of 4 isolates tested, respectively. None of
the patients with CDAD due to type 027 had severe disease.

A cluster of 12 patients with CDAD by PCR ribotype
027, toxino type III, was reported in July and August in a
large teaching hospital in Amsterdam (hospital 5). One
patient died from consequences of CDAD, and severe
complications developed in 2 other patients. Another hos-
pital in Amsterdam (hospital 6) also reported an increase of

<table>
<thead>
<tr>
<th>Hospital no. and setting</th>
<th>No. beds</th>
<th>Incidence/10,000 before outbreak</th>
<th>Maximum incidence/mo/10,000 during outbreak†</th>
<th>Date of outbreak onset</th>
<th>Total no. CDAD patients in given period, 2005</th>
<th>Deaths, 30 d</th>
<th>No. strains studied</th>
<th>No. toxinotype III, PCR ribotype 027 strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Harderwijk</td>
<td>341</td>
<td>4</td>
<td>83</td>
<td>Apr 2005</td>
<td>51, Apr–Nov</td>
<td>3</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>2. Amersfoort</td>
<td>600</td>
<td>11</td>
<td>87</td>
<td>May 2005</td>
<td>85, Jan–Dec</td>
<td>19</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>3. Utrecht</td>
<td>1,013</td>
<td>–</td>
<td>No outbreak</td>
<td>37, Jun–Dec</td>
<td>Unk.</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4. Nieuwegein</td>
<td>584</td>
<td>–</td>
<td>No outbreak</td>
<td>13, Jan–Dec</td>
<td>Unk.</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5. Amsterdam</td>
<td>1,002</td>
<td>38</td>
<td>52</td>
<td>68, Jan–Oct</td>
<td>28</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Amsterdam</td>
<td>310</td>
<td>10</td>
<td>66</td>
<td>Apr–May 2005</td>
<td>42, Jan–Oct</td>
<td>Unk.</td>
<td>34</td>
<td>16</td>
</tr>
<tr>
<td>8. Hoofddorp</td>
<td>455</td>
<td>3</td>
<td>76</td>
<td>Jan 2005</td>
<td>73, Jan–Dec</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

*PCR, polymerase chain reaction; CDAD, Clostridium difficile–associated diarrhea; unk., unknown.
†Timeframe 2–4 mo.
The observation that outbreaks due to different strains can occur simultaneously emphasizes that microbiologic monitoring is important for epidemiologic studies of CDAD. PCR ribotype 017 strain lacks a part of the toxin A gene and was first recognized as a cause of an outbreak in Canada in 1999 (14). Subsequently, toxin A–negative, toxin B–positive strains caused outbreaks of CDAD in Ireland (D. Drudy, pers. comm.), Argentina (M.C. Legaria, et al., unpub. data), and the Netherlands (15).

The outbreaks in the Netherlands are difficult to control. In the Harderwijk epidemic, using rapid diagnostic tests for CDAD and cohort isolation in combination with restricting use of fluoroquinolones and cephalosporins appeared to be successful. Outbreaks in the other hospitals are still not completely under control.

Acknowledgments

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Dr Kuijper is a medical microbiologist at Leiden University Hospital. His research interests include C. difficile infections and emerging bacterial infections.

References


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