Relapse Versus Reinfection: Surveillance of Clostridium difficile Infection

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Molecular typing was used to examine surveillance definitions for recurrent Clostridium difficile–associated diarrhea. Among 102 patients, 85 had a second episode within 8 weeks, 88% of which were relapses. Of 49 second episodes occurring after >8 weeks, 65% were relapses. Categorization of a recurrent episode occurring after >8 weeks as a new infection may misrepresent the majority of episodes for surveillance.

In recent years, Clostridium difficile infection (CDI) has become more frequent, severe, and difficult to treat [1–3]. It is now recommended that healthcare facilities perform active surveillance for CDI, and many states in the United States have mandated facility-specific public reporting of healthcare-associated CDI rates [4]. To establish a uniform approach to surveillance, the Centers for Disease Control and Prevention constituted an Ad Hoc C. difficile Surveillance Working Group to develop interim surveillance definitions for new, recurrent, and healthcare facility–associated CDI. A particular challenge is classification of persons who have >1 episode of CDI. For these patients, the Ad Hoc Working Group determined that a second episode of CDI should be categorized as either a relapse or a second new infection (reinfection), based on the interval between positive test results. Specifically, they recommended that a relapse be defined as a second episode occurring within 2–8 weeks of the index case; a second new episode was defined as occurring ≥8 weeks after the index case [4].

Polymerase chain reaction (PCR) ribotyping, based on polymorphisms in the 16S-23S ribosomal RNA interspacer region, is a rapid method for typing of C. difficile isolates with good discriminatory power [5]. It is easy to perform and highly reproducible and is widely used in Europe for hospital-based surveillance of CDI [6]. In this study, we applied PCR ribotyping to specimens from a series of patients with ≥2 episodes of CDI to determine whether the second episode was due to the same strain as the first or a different strain. We then consider these results in the context of the Ad Hoc Working Group’s current recommendations [4]. The accuracy of surveillance definitions is increasingly important given the public scrutiny of healthcare-associated conditions and the possibility that reimbursement eventually may be decreased when patients develop a healthcare-associated infection such as CDI.

METHODS

Memorial Sloan-Kettering Cancer Center is a 470-bed tertiary care cancer center in New York City. Between January 2008 and June 2010, patients with ≥2 episodes of CDI occurring ≥2 weeks apart were identified by means of microbiology records, and the intervals between episodes were determined. Patients with >1 recurrence (≥3 episodes) were examined by intervals between first and second then second and third episodes; the time from the first to the third episode was not analyzed. Patients who had had episodes before the start of the study period were excluded. The institutional review board reviewed the study and granted a Health Insurance Portability and Accountability Act waiver of authorization.

Diagnosis of Clostridium difficile Infection

Before September 2009, all stool samples submitted to the Microbiology Laboratory were evaluated by using the C. difficile cytotoxin neutralization assay. After September 2009, a 2-step algorithm was implemented, using an enzyme immunoassay for the detection of C. difficile common antigen glutamate dehydrogenase as a screening assay, followed by testing of all glutamate dehydrogenase–positive stools with the cytotoxin neutralization assay. Isolates were studied by PCR ribotyping, as described elsewhere by Bidet et al [5]. The American Type Culture Collection strain BAA-1805 (C. difficile, North American pulse-field type 1 [NAP1]; toxino type III, binary toxin positive) was included as a reference strain in all gels.

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Statistical Analysis
Statistical analysis was performed using Fisher’s exact and $\chi^2$ tests and GraphPad Quick Calcs software. Microsoft Excel graphing (linear regression) was used to calculate the slope, y-intercept, and correlation coefficient for data shown in the Figure 1.

RESULTS
The incidence of CDI during the 30-month study period was 21 cases/10,000 patient days, and the CDI rate of hospital-acquired disease was 9.8 cases/10,000 patient days. A total of 134 paired isolates of *C. difficile* from 102 patients were analyzed by PCR ribotyping. Among the 134 pairs, 85 were collected 2–8 weeks apart, and 49 were collected >8 weeks apart. Twenty-four patients (24%) had ≥3 episodes of CDI. No outbreaks were detected by molecular typing during the study period.

Episodes of *Clostridium difficile* Infection >2 and <8 Weeks Apart
Eighty-five paired isolates of *C. difficile* from 70 patients were analyzed by PCR ribotyping. The mean interval between these episodes was 28 days, and the median was 25 days (range, 14–55 days). For 75 (88%) of 85 episodes, the second strain was identical to the original infecting strain, reflecting relapse. The likelihood of having a second infection did not vary for pairs collected <4 or >4 weeks apart (90% vs 86.5%) (Table 1). Limiting the analysis to comparison only between the index case and the second episode (ie, excluding the third and fourth episodes) did not change the results (Table 1).

<table>
<thead>
<tr>
<th>Interval between episodes, weeks</th>
<th>Total</th>
<th>Relapse</th>
<th>Second infection</th>
<th>Total</th>
<th>Relapse</th>
<th>Second infection</th>
</tr>
</thead>
<tbody>
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<td></td>
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<tr>
<td>2–4</td>
<td>48</td>
<td>43 (90)</td>
<td>5 (10)</td>
<td>37</td>
<td>32 (86.5)</td>
<td>5 (13.5)</td>
</tr>
<tr>
<td>4–8</td>
<td>37</td>
<td>32 (86.5)</td>
<td>5 (13.5)</td>
<td>28</td>
<td>24 (86)</td>
<td>4 (14)</td>
</tr>
<tr>
<td>&gt;8</td>
<td>49</td>
<td>32 (65)</td>
<td>17 (35)</td>
<td>37</td>
<td>24 (65)</td>
<td>13 (35)</td>
</tr>
</tbody>
</table>
proportion of patients with true relapse, as characterized by
studies to distinguish relapse from second new infection. The
usually have relapses from this strain rather than second in-
had symptomatic
the median 98 days (range, 56–337 days). None of the patients
analyzed. The mean interval between episodes was 125 days, and
Among the 24 patients with
$3$ episodes, weeks
Interval between episodes, no. (%) NAP1 Non-NAP1
≤8 25/25 (100) 52/60 (87) .05
>8 8/10 (80) 24/39 (62) .23
Episodes of Clostridium difficile Infection ≥8 Weeks Apart
Forty-nine paired isolates of C. difficile from 48 patients were
analyzed. The mean interval between episodes was 125 days, and
the median 98 days (range, 56–337 days). None of the patients
had symptomatic C. difficile infection between the episodes ex-
amined. For 32 (65%) of 49 cases, PCR ribotyping showed
identical strains for both episodes. As with episodes recurring
within 8 weeks, exclusion of the third and fourth episodes did
not change the results (Table 1; Figure 1).

Impact of North American Pulse-Field Type 1 Strain
The proportion of cases due to the NAP1 strain varied widely
during the study period. A 3-month prevalence survey done
from June to August 2008 showed a 40% prevalence of NAP1.
More recently, a 10-week study from March to May 2010
showed that the prevalence of NAP1 had decreased to 16%.
Exclusion of NAP1 isolates from the study did not change the
findings (Table 2).

Multiple Episodes of Clostridium difficile Infection
Among the 24 patients with ≥3 episodes of CDI, 17 had
≥4 episodes. The mean duration between the first and second
episodes was 7.3 weeks, and the mean duration between the
second and third episodes was 9.8 weeks. Eleven of 17 patients
(65%) had the same strain isolated for all episodes. Exclusion of
the third and fourth episodes from the analysis did not change
the results.

DISCUSSION
We examined C. difficile isolates from 102 patients with repeated
episodes of CDI. Our findings suggest that for those with a sec-
ond episode within 8 weeks of the index case, almost all second
episodes are due to the same strain. However, even for episodes
occurring >8 weeks after the initial episode, the majority (65%) are
also due to the original infecting strain and thus represent
relapse and not a second new infection (Figure 1). Our study
also suggests that patients with initial infection due to NAP1
usually have relapses from this strain rather than second in-
fecions from another strain.

Molecular typing has been used in 6 previously published
studies to distinguish relapse from second new infection. The
proportion of patients with true relapse, as characterized by
DNA fingerprinting, has varied from 25% to 67% in these
reports [7–12]. Most of the studies were performed before the
emergence of the NAP1 strain and are limited by relatively
small sample sizes. In addition, only a small fraction of
multiple isolates analyzed represent episodes that occurred
>8 weeks apart.

Distinguishing relapses from second new infections by means of
PCR ribotyping may have certain limits. Recently, Sethi et al
used culture and typing to prospectively follow up 52 patients
treated for CDI for up to 1–4 weeks after treatment [13]. They
observed that more than half of these patients became asym-
ptomatic carriers of C. difficile, and the strain type isolated from
a patient’s environment and/or skin usually matched that
causing CDI. They posited that asymptomatic shedding of
C. difficile could contribute to horizontal transmission. This
pathophysiologic explanation would represent a true “new
infection” that, on ribotyping with our methods, would appear to
be a relapse rather than a new infection and fundamentally
change the interpretation of our findings. However, the con-
tribution of this possible route of disease development is not yet
settled.

Our study has several limitations. First, we did not use the
most discriminatory typing methods to determine genetic re-
latedness between strains. We did not assess for clinical resolu-
tion of symptoms between episodes. However, we adopted this
study methodology to follow the laboratory-based reporting
system proposed by the Surveillance Working Group and chose
PCR ribotyping because of its ease of performance and potential
for widespread use as an epidemiologic tool.

Finally, the contribution of NAP1 cannot be fully determined;
this strain has a higher rate of therapeutic failure, so relapse may
appear more frequent. Our findings, unlike those of the previous
6 reported studies, may have influenced by the presence of the
NAP1 strain. Most hospitals now have endemic NAP1 disease;
our hospital and findings may therefore be more representative
of the actual dynamics of CDI in the 21st century. Exclusion of
NAP1 strains from the results, however, did not change our
conclusions.

According to the proposed definitions by the Ad Hoc
C. difficile Surveillance Working Group, second CDI episodes
that occur >8 weeks after the index case should be classified as
second infections from a new strain. However, we found that
the majority of these cases are in fact due to relapse from the same
strain causing the index episode. Such a distinction is important:
in the era of public reporting, hospital rates of CDI are being
monitored closely. Misclassification of a case as a “new case” will
artificially inflate hospital rates of disease, resulting in inaccurate
data and, possibly, deleterious economic effects for institutions.
It is crucial therefore to base surveillance definitions on evi-
dence, including molecular typing results from patients with
multiple episodes of CDI.

Table 2. Relapse Stratified by Strain Type of Original Infecting
Clostridium difficile Isolates (North American Pulse-Field Type 1
[NAP1] Versus Non-NAP1)

<table>
<thead>
<tr>
<th>Interval between episodes, weeks</th>
<th>Same strain at relapse/</th>
<th>Total infections, no. (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤8</td>
<td>NAP1</td>
<td>Non-NAP1</td>
<td></td>
</tr>
<tr>
<td>25/25 (100)</td>
<td>52/60 (87)</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>&gt;8</td>
<td>8/10 (80)</td>
<td>24/39 (62)</td>
<td>.23</td>
</tr>
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Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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References