Does Infection With Specific Clostridium difficile Strains or Clades Influence Clinical Outcome?

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(See the Major Article by Walker et al on pages 1589–600.)

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A number of DNA banding–based typing systems for classifying Clostridium difficile isolates have been devised including polymerase chain reaction (PCR) ribotyping, restriction endonuclease analysis (REA), and pulsed-field gel electrophoresis (PFGE) [1–4]. PCR ribotyping was developed in Wales and is commonly used in Europe, whereas REA and PFGE have been used more commonly in North America. In addition, toxinotyping, which is based on amplification of regions within the pathogenicity locus (PaLoc) for toxins A and B, has been used to describe “toxin variant” strains whose PaLoc genes differ from the standard or toxinotype “0” PaLoc to which most PCR ribotypes, REA groups, and PFGE types belong [5]. The aforementioned DNA-banding typing systems are invaluable for discriminating isolates from each other, but do not provide meaningful evolutionary analyses.

Multilocus sequence typing (MLST), in which a selected number of housekeeping gene loci (7 in the case of C. difficile) are amplified and sequenced to yield sequence types (STs), can be used to group STs by evolutionary relationships into clades [6]. Members of a clade are considered to be derived from the same common ancestor and can be organized into evolutionary trees using phylogenetic or cladistic analyses. MLST classification of C. difficile clades agrees with previous classification based on DNA microarray comparative genomics and suggests that genotypes clustered by MLST may be an accurate proxy for whole-genome sequencing analysis [7]. STs correlate well with PCR ribotypes; however, as with toxinotyping, the vast majority of STs are clustered in a single clade, clade 1, with only 1 or 2 STs (ribotypes) in each of the remaining 4 clades (Table 1).

Since the description of the epidemic NAP1/BI/027 strain of C. difficile as the cause of increased rates of C. difficile infection (CDI), there has been an ongoing debate over whether these strains are responsible for increased severity of CDI including increased mortality [3, 4]. Clearly, the incidence of CDI and associated mortality have increased temporally in the United States with the increased frequency of the NAP1/BI/027 strain [13]. In Canada, Miller et al examined CDI severity (as measured by intensive care unit [ICU] admission for CDI, colectomy due to CDI, or CDI-attributable death) in 1008 CDI patients from multiple national sites and found a highly significant association (P < .001) with infection caused by the NAP1 C. difficile strain and age of the patient. More recently, the association between PCR ribotypes (027 and 078 considered together) and severe CDI was challenged by Walk et al using separate derived and validated data sets from one hospital in Michigan [14]. Their major finding was that the association of severe CDI (ICU admission, interventional surgery, or 30-day mortality) with PCR ribotypes 027 and 078 was not significant after adjustment for covariates, and furthermore, that severe CDI was predicted only by patient biomarkers of white blood cell (WBC) count and serum albumin level.

The pendulum has swung back toward the importance of organism type with the article in this issue of Clinical Infectious Diseases by Walker et al, who also considered the relationship between bacterial strain type, host biomarkers, and 14-day CDI mortality occurring over a span of nearly 5 years in 4 hospitals in Oxfordshire, United Kingdom [8]. They based their analysis on a combination of PCR ribotyping and MLST typing, which we have attempted to correlate with prior typing methods (Table 1). In contrast to Walk et al, Walker et al found significantly increased 14-day mortality (adjusted P < .0001) of 25% for ST clade 5 (ribotype 078), 20% for ST clade 2...
Table 1. Multilocus Sequence Typing (MLST), Sequence Type (ST), Polymerase Chain Reaction Ribotypes, Restriction Endonuclease Analysis Groups, Pulsed-Field Gel Electrophoresis Types, Toxinotypes, and Presence of Binary Toxin in a Selected Group of *Clostridium difficile* Toxigenic Strains Found in Different MLST STs

<table>
<thead>
<tr>
<th>MLST Clade</th>
<th>Sequence Type</th>
<th>PCR Ribotype</th>
<th>REA Group</th>
<th>PFGE Type</th>
<th>Toxinotype</th>
<th>Binary Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ST3</td>
<td>001</td>
<td>J</td>
<td>NAP2</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>1</td>
<td>ST8</td>
<td>002</td>
<td>G</td>
<td>NAP8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>1</td>
<td>ST10</td>
<td>015</td>
<td>N</td>
<td>NAP12</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>1</td>
<td>ST2</td>
<td>014, 020</td>
<td>Y</td>
<td>NAP4</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>1</td>
<td>ST42</td>
<td>106</td>
<td>DH</td>
<td>NAP11</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>ST1</td>
<td>027</td>
<td>BI</td>
<td>NAP1</td>
<td>III</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>ST5, ST22</td>
<td>023</td>
<td>AN</td>
<td>Unnamed</td>
<td>IV</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>ST37</td>
<td>017</td>
<td>CF</td>
<td>NAP9</td>
<td>VIII</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>ST11</td>
<td>078, 126</td>
<td>BK</td>
<td>NAP7, 8, 9</td>
<td>V</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The vast majority of *Clostridium difficile* strains are found in MLST clade 1 and toxinotype 0. Data derived from references [8–12] and personal communication from Duncan MacCannell, Centers for Disease Control and Prevention, Atlanta, Georgia. Abbreviations: MLST, multilocus sequence typing; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; REA, restriction endonuclease analysis; ST, sequence type.

*Less than 80% agreement with PCR ribotype.*

(ribotype 027), and 12% for ST clade 1 (multiple ribotypes) [8, 14]. In agreement with Walk et al, they also found strong associations with biomarkers and mortality including WBC counts, serum albumin, C-reactive protein (CRP), and eosinophil counts, but also found the biomarkers to be associated with organism clades and to predict an estimated 30%–40% of clade-specific mortality [8, 14].

How can the differences between these 2 recent publications be reconciled? The most critical difference is likely the number of CDI cases analyzed in the 2 studies. Walker et al included 1893 toxin enzyme immunoassay (EIA) and culture-positive strain-typed cases in their analysis, whereas Walk et al had only 310 such cases in their derivation set and 433 in their validation data set [8, 14]. The 2 studies also differed in their study endpoints: CDI severity for Walk et al (ICU admission, interventional surgery, or 30-day mortality), and 14-day mortality for Walker et al. Walk et al combined ribotypes 027 and 078, whereas Walker et al analyzed ribotypes 027, 078, and 023 separately. CDI was diagnosed on the basis of toxin EIA testing by Walker et al. The laboratory test used clinically by Walk et al was either EIA or *tcdB* gene PCR, the latter more sensitive than EIA and possibly detecting patients with *C. difficile* colonization whose diarrhea symptoms were from another cause [15]. EIA testing is notoriously insensitive, and the practice of sending multiple stools for EIA testing also leads to false-positive results, which was at least partly confirmed by the finding of EIA-positive stools that were culture negative, a potential criticism of the study of Walker et al.

Additional studies confirm the importance of strain type or strain attributes on CDI outcome. Hensgens et al [16] found increased 30-day mortality in the Netherlands during a nonepidemic period associated with ribotype 027 (*P* = .04) when adjusted for age and sex of the patients, despite the fact that it was the fourth most commonly isolated ribotype; however, there was no increased mortality from ribotype 078, the second most frequently isolated ribotype. Adjustment for biomarkers was not done. Because ribotypes 027 and 078 both contain binary toxin (Table 1), there has been interest in whether binary toxin could be a common attribute of strains associated with increased mortality. Goldenberg and French [17] in a UK study found that patients infected with isolates containing *tcdC* truncating mutations had significantly elevated CRP and peripheral WBC counts compared with patients not infected with these strains, but there was no difference in patient outcome. In contrast, patients infected with *C. difficile* strains containing binary toxin not only had significantly higher WBC, but also had a significantly higher 30-day all-cause mortality (31% vs 14%; *P* = .02). Only 8% of isolates were ribotype 027, whereas 28% of isolates contained binary toxin genes. Similarly, Bacci et al [18] studied isolates forwarded to the National Reference Laboratory at Statens Serum Institut (Copenhagen, Denmark) that were resistant to moxifloxacin, had a severe clinical course, or were suspected in a CDI outbreak. They compared 212 *C. difficile* isolates that were toxin A*/B* to 265 isolates that were toxin A*/B*/* binary toxin*, including 193 isolates of PCR ribotype 027 and 72 isolates that were non-027 (ribotypes 078, 066, and 023). Thirty-day mortality after diagnosis was 28% for PCR ribotype 027 strains, and 27.8% for binary toxin-positive non-027 isolates compared to 17% for patients infected with A*/B* isolates. Multivariate analysis, after adjustment for age, sex, and region, showed that the relative risk
was 1.6 (95% confidence interval, 1.0–2.4) for case patients infected with the A’/B’/binary toxin− strains compared with the patients infected with A’/B’ strains lacking binary toxin genes. These intriguing observations implicating binary toxin as a virulence factor associated with increased CDI mortality are not supported by the observations of Walker et al, who found that ribotype 023, which is a binary toxin−positive strain, was associated with increased WBC and CRP, but not increased mortality [8].

Despite clear associations of C. difficile strain type with increased mortality, most patients tolerate infection with these strains well, demonstrating both asymptomatic colonization and mild CDI [19, 20]. Thus, rather than basing treatment strategies on strain type, we agree with previous commentators who recommended that clinical scores or biomarkers for CDI severity continue to be the basis for treatment decisions [21, 22]. Several biomarkers identified in these recent publications, including CRP, and albumin level in addition to WBC, deserve further assessment as CDI severity predictors. Further delineation of the importance of binary toxin as a common virulence factor in C. difficile requires additional confirmation, especially PCR ribotype 023, which demonstrates strong correlation with biomarkers but unlike ribotypes 027 and 078, not with increased mortality.

Notes

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Potential conflicts of interest. D. N. G. holds patents for the treatment and prevention of Clostridium difficile infection licensed to ViroPharma, and is a consultant for Merck, ViroPharma, Pfizer, GlaxoSmithKline, Roche, Novartis, Optimer, Cubist, BioRelix, Cangene, Medicines Co, and Actelion and holds research grants from GOJO and Sanofi Pasteur. S. J. has served as a consultant for Optimer, Pfizer, and Bio-K+.

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References