Clostridium difficile Ribotype 027: Relationship to Age, Detectability of Toxins A or B in Stool With Rapid Testing, Severe Infection, and Mortality

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(See the Editorial Commentary by Goorhuis on pages 242–3.)

Background. Clostridium difficile infection (CDI) can cause severe disease and death, especially in older adults. A better understanding of risk factors for adverse outcomes is needed. This study tests the hypotheses that infection with specific ribotypes and presence of stool toxins independently associate with severity and constructs predictive models of adverse outcomes.

Methods. Cases of non-recurrent CDI were prospectively included after positive stool tests for toxins A and/or B by enzyme immunoassay (EIA) or tcdB by polymerase chain reaction. Outcomes included severe CDI (intensive care unit admission, colectomy, or death attributable to CDI within 30 days of diagnosis) and 30-day all-cause mortality. Adjusted models were developed to test hypotheses and predict outcomes.

Results. In total, 1144 cases were included. The toxin EIA was positive in 37.2% and 35.6% of patients were of age >65 years. One of the 137 unique ribotypes was ribotype 027 (16.2%). Detectable stool toxin did not associate with outcomes. Adjusting for covariates, including age, Ribotype 027 was a significant predictor of severe CDI (90 cases; odds ratio [OR], 1.73; 95% confidence interval [CI], 1.03–2.89; P = .037) and mortality (89 cases; OR, 2.02; 95% CI, 1.19–3.43; P = .009). Concurrent antibiotic use associated with both outcomes. Both multivariable predictive models had excellent performance (area under the curve >0.8).

Conclusions. Detection of stool toxin A and/or B by EIA does not predict severe CDI or mortality. Infection with ribotype 027 independently predicts severe CDI and mortality. Use of concurrent antibiotics is a potentially modifiable risk factor for severe CDI.

Keywords. Clostridium difficile infection; colitis; ribotype; risk prediction models.
outcomes such as all-cause mortality [5]. It remains controversial whether ribotype 027 is hypervirulent—isolates with the same ribotype can have different phenotypes, such as the degree of toxin production [6]; ribotype 027 strains derive from 2 distinct genetic lineages [7]; clinical studies on the topic have reached different conclusions [4, 8, 9]; and a tight epidemiologic link has been demonstrated between ribotype 027 infection and advanced age, itself an important risk factor for mortality [10].

Older adults are disproportionately affected by CDI, with the annual rate of CDI-related hospitalizations per 100,000 individuals in those aged >85 years exceeding rate for all other age groups combined [11]. Furthermore, older adults are at the highest risk of adverse outcomes related to CDI. Ninety-two percent of CDI-related deaths occur in those aged >65 years; in this population, CDI is the 18th leading cause of death [3]. Since outcomes in older adults are largely driven by increased frailty, decreased functional status, and a higher burden of comorbid disease [12–15], the role that any intrinsic virulence of ribotype 027 plays remains uncertain [11].

Rapid tests for diagnosis vary but usually detect the toxins A and/or B directly in stool by enzyme immunoassay (EIA) or the gene for toxin B, tcdB, by real-time PCR. The relative importance of patient demographics, clinical features of infection, ribotype, bacterial load, and detectability of toxin in stool by rapid tests have yet to be determined. This study has 2 broad aims: to test the hypothesis that PCR ribotype and detectability of stool toxin by EIA are independently associated with severe CDI and mortality in hospitalized adults, even after adjustment for age and other potential confounders, and to develop predictive models for severe CDI and mortality.

MATERIALS AND METHODS

Patients

The University of Michigan Institutional Review Board approved this study. Stool samples from hospitalized patients at the University of Michigan Health System that were submitted to the microbiology laboratory for C. difficile testing in patients aged ≥18 years and not pregnant were consecutively evaluated for inclusion between October 2010 and January 2013. All included samples tested positive for the presence of toxigenic C. difficile. All laboratory testing of inpatients was performed at the discretion of the inpatient care team, which ordered C. difficile testing per institutional guidelines that mirror national guidelines that recommend testing of only symptomatic patients with suspected CDI [16, 17]. Samples were sent to the laboratory in Cary-Blair transport medium, per hospital policy. Positive samples from the same patient within 8 weeks of the index sample were excluded, as they were not thought to represent a separate or new episode of CDI.

Microbiology

Testing was performed on stool samples via an algorithm (Figure 1) using the C. DIFF QUIK CHEK COMPLETE test for C. difficile glutamate dehydrogenase (GDH) and toxins A or B (Techlab, Inc., Blacksburg, Virginia) by EIA. All GDH+/toxin− stool tests were subjected to analysis for the tcdB gene by real-time PCR using the GeneOhm Cdiff Assay (BD, Franklin Lakes, New Jersey) run on a Cepheid SmartCycler System (Cepheid, Sunnyvale, California). Confirmation of all positive C. difficile tests was attempted by anaerobic culture on taurocholate–cycloserine–cefoxitin–fructose agar at 37°C. Attempts were made to ribotype samples using high-throughput, fluorescent PCR ribotyping as described elsewhere [8, 18].

Data Extraction

Data regarding demographics, comorbid disease (taken from International Classification of Diseases, Ninth Revision [ICD-9] codes), vitals, laboratory test results, medications, and outcomes were extracted from the electronic medical record through structured query. Values for vitals and laboratory results were included if available within 24–48 hours of diagnosis. Where available, PCR cycle threshold (C_T) values were obtained through query of the Cepheid SmartCycler software database. An unweighted Charlson-Deyo comorbidity score was calculated from ICD-9 codes [19]. Severe CDI was defined as occurrence of any of the following outcomes attributable to CDI within 30 days of diagnosis: admission to an intensive care unit, the need for intraabdominal surgery (such as colectomy), or death [20].

Bivariable relationships were assessed using simple logistic regression. Ribotype was initially modeled as a categorical variable; however, based on the results (discussed below), it was reconstructed as a binary variable for ribotype 027. The variables serum creatinine, total bilirubin, and platelet count were dichotomized. The primary outcome was severe CDI, and the secondary outcome was...
30-day all-cause mortality. Due to the importance of ribotype 027 seen in our data (see results below), it was adjusted individually with other covariates in relation to severe CDI and mortality.

To address the first broad study aim and test the hypotheses that ribotype and detectability of toxin by EIA associate with severe CDI and mortality, multivariable logistic regression models that included variables not in the causal pathway (demographics, comorbid disease, and medications) were developed. To address the second study aim of developing predictive models for adverse outcomes from CDI, models for the primary and secondary outcomes were developed using all available variables. Stepwise addition and backward elimination using the likelihood ratio test (threshold of \( P < .1 \)) were used to construct initial candidate models, which were modified after consideration of other variables with statistical or a priori study aim significance. Model fit was assessed with attention to the Hosmer–Lemeshow test implemented in the R package ResourceSelection (Subhash et al 2014), presence of overdispersion, and variable coefficients. Interaction terms were assessed. Predictive models were additionally evaluated using receiver operator characteristic (ROC) curves from the R package \( \text{pROC} \) [22].

### RESULTS

#### Population Characteristics

Samples from 1144 discrete episodes of CDI from 981 individuals met study criteria for inclusion (Figure 1). Of cases, 37% were diagnosed by a positive toxin A or B EIA test and the remainder by a positive PCR for \( tcdB \). Notably, no cases were GDH−/toxin+.

Selected study population characteristics are summarized in Table 1. Older adults (aged >65 years) comprised 35.6% of cases; 71% of cases were healthcare associated (onset of symptoms >48 hours after admission); and concurrent non-CDI antibiotic use occurred in 66%. The \( C. \text{difficile} \) isolates had significant genetic diversity, with 137 distinct ribotypes identified. There were more isolates of ribotype 014-020 (16.2%) and ribotype 027 (16.2%) than any other single ribotype. Forty-five isolates (3.9%) could not be ribotyped due to inadequate growth of \( C. \text{difficile} \) or insufficient remaining sample.

#### Analysis of Missing Data

Values were missing for a number of variables (Table 1). Except for albumin and bilirubin, missingness was not associated with

![Figure 1.](image-url)  
The University of Michigan diagnostic testing algorithm for detecting toxigenic \( C. \text{difficile} \) in stool. The number of cases from this study relevant to each step is shown. Abbreviations: CDI, \( C. \text{difficile} \) infection; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; PCR, polymerase chain reaction.
severe CDI or mortality, and case-wise deletion was used in further analyses. Missing albumin or bilirubin values were associated with both severe CDI (odds ratio [OR], 0.38; 95% confidence interval [CI], .18–.80; \(P = .011\)) and mortality (OR, 0.50; 95% CI, .26–.99; \(P = .046\)). One-way sensitivity analyses over plausible laboratory ranges showed that the associations did not change in direction or significance. Thus, missing albumin and bilirubin values were imputed.

**Initial Bivariable Relationships**

Detectable toxin and/or ribotype 027 associated with age, Charlson-Deyo score, prior CDI (defined as an episode of CDI >8 weeks prior), HA CDI, congestive heart failure (CHF), diabetes mellitus, proton pump inhibitor (PPI) use, bilirubin value, and white blood cell count (WBC); detectable toxin and ribotype 027 were also associated with each other (data not shown). Thus, these variables were scrutinized during modeling.

**Study Aim 1: Associations With Primary and Secondary Outcomes**

Severe CDI occurred in 7.9% of patients and death occurred within 30 days in 7.8% of patients. Selected results from the unadjusted analysis are shown in Table 2. Notably, the following associated with severe CDI: age, metastatic cancer, CHF, depression, concurrent antibiotic use, PPI use, systolic blood pressure, WBC, bilirubin value, and ribotype 027 (but not other ribotypes). Thirty-day all-cause mortality was also associated with many of these same variables, most notably, prior CDI and ribotype 027 (but not other ribotypes). A detectable toxin and/or ribotype associated with age, Charlson-Deyo score, prior CDI (defined as an episode of CDI >8 weeks prior), HA CDI, congestive heart failure (CHF), diabetes mellitus, proton pump inhibitor (PPI) use, bilirubin value, and white blood cell count (WBC); detectable toxin and ribotype 027 were also associated with each other (data not shown). Thus, these variables were scrutinized during modeling.

**Study Aim 2: Multivariable Predictive Models**

The final multivariable predictive models selected for severe CDI and mortality are shown in Table 4. These were selected due to their superior predictive ability and fit with the data compared with competing models. Interaction terms were not significant. Age, metastatic cancer, and concurrent use of antibiotics were important predictors in both models. The final models for severe CDI and mortality both had area under the curves >0.8 and nonsignificant Hosmer–Lemeshow tests, suggesting good fit (Figures 2 and 3).
DISCUSSION

This study tested the hypothesis that infection with ribotype 027 and detectable stool toxin are independently associated with severe CDI and mortality; it also developed multivariable predictive models with good fit characteristics. The results provide evidence for an independent association between infection with ribotype 027 and both severe CDI and 30-day all-cause mortality.

Table 2. Unadjusted Analysis of Selected Predictors for Severe *Clostridium difficile* Infection and 30-Day All-Cause Mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severe CDI</th>
<th>P Value</th>
<th>30-Day Mortality</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>1.03 (1.01–1.04)</td>
<td>&lt;.001</td>
<td>1.03 (1.02–1.05)</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>Female gender</td>
<td>1.4 (0.90–2.19)</td>
<td>.133</td>
<td>0.88 (0.57–1.36)</td>
<td>.561</td>
<td></td>
</tr>
<tr>
<td>White race</td>
<td>0.72 (0.42–1.23)</td>
<td>.23</td>
<td>0.98 (0.55–1.75)</td>
<td>.943</td>
<td></td>
</tr>
<tr>
<td>Charlson-Deyo score</td>
<td>1.06 (0.95–1.2)</td>
<td>.297</td>
<td>1.13 (1.01–1.27)</td>
<td>.031</td>
<td></td>
</tr>
<tr>
<td>Prior CDI</td>
<td>0.67 (0.39–1.15)</td>
<td>.144</td>
<td>0.54 (0.3–0.95)</td>
<td>.033</td>
<td></td>
</tr>
<tr>
<td>Healthcare-associated CDI</td>
<td>0.75 (0.48–1.19)</td>
<td>.223</td>
<td>1.66 (0.97–2.82)</td>
<td>.064</td>
<td></td>
</tr>
<tr>
<td>Solid organ neoplasm</td>
<td>1.27 (0.73–2.21)</td>
<td>.393</td>
<td>1.74 (1.04–2.92)</td>
<td>.036</td>
<td></td>
</tr>
<tr>
<td>Metastatic cancer</td>
<td>2.46 (1.2–5.03)</td>
<td>.014</td>
<td>4 (2.11–7.6)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Liver disease</td>
<td>1.41 (0.79–2.53)</td>
<td>.25</td>
<td>1.98 (1.16–3.4)</td>
<td>.013</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.54 (0.99–2.38)</td>
<td>.053</td>
<td>1.42 (0.92–2.21)</td>
<td>.118</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>1.87 (0.90–3.2)</td>
<td>.023</td>
<td>3.07 (1.87–5.03)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.86 (0.52–1.43)</td>
<td>.567</td>
<td>0.66 (0.38–1.13)</td>
<td>.131</td>
<td></td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>1.47 (0.93–2.31)</td>
<td>.1</td>
<td>1.58 (1.00–2.48)</td>
<td>.049</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>1.64 (1.02–2.66)</td>
<td>.043</td>
<td>1.13 (0.67–1.9)</td>
<td>.639</td>
<td></td>
</tr>
<tr>
<td>Concurrent antibiotic use</td>
<td>6.71 (3.07–14.7)</td>
<td>&lt;.001</td>
<td>3.94 (2.07–7.51)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Prior fluoroquinolone use</td>
<td>1.05 (0.67–1.65)</td>
<td>.84</td>
<td>0.72 (0.44–1.16)</td>
<td>.177</td>
<td></td>
</tr>
<tr>
<td>Proton pump inhibitor use</td>
<td>2.37 (1.34–4.19)</td>
<td>.003</td>
<td>1.71 (1.02–2.89)</td>
<td>.044</td>
<td></td>
</tr>
<tr>
<td>Fever (&gt;38°C)</td>
<td>1.9 (1.19–3.04)</td>
<td>.007</td>
<td>1.29 (0.78–2.13)</td>
<td>.329</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>0.97 (0.96–98)</td>
<td>&lt;.001</td>
<td>0.98 (0.96–99)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>4.44 (2.83–6.95)</td>
<td>&lt;.001</td>
<td>4.53 (2.89–7.11)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Sodium (mg/dL)</td>
<td>0.94 (0.90–99)</td>
<td>.016</td>
<td>0.94 (0.90–99)</td>
<td>.021</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.19 (0.9–1.3)</td>
<td>&lt;.001</td>
<td>1.15 (0.91–1.27)</td>
<td>.003</td>
<td></td>
</tr>
<tr>
<td>Creatinine &gt;1.5 mg/dL</td>
<td>3.37 (2.18–5.22)</td>
<td>&lt;.001</td>
<td>2.83 (1.83–4.39)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>0.4 (0.28–0.58)</td>
<td>&lt;.001</td>
<td>0.41 (0.28–0.59)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Imputed albumin (g/dL)</td>
<td>0.38 (0.26–0.54)</td>
<td>&lt;.001</td>
<td>0.37 (0.26–0.53)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>1.06 (1.02–1.11)</td>
<td>.003</td>
<td>1.09 (1.05–1.14)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Imputed total bilirubin (mg/dL)</td>
<td>1.07 (1.03–1.11)</td>
<td>.001</td>
<td>1.1 (1.06–1.15)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin &gt;1.2 mg/dL</td>
<td>2.29 (1.43–3.67)</td>
<td>.001</td>
<td>4.03 (2.57–6.32)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>White blood cell count (cells/µL)</td>
<td>1.03 (1.02–1.05)</td>
<td>&lt;.001</td>
<td>1.03 (1.01–1.04)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>0.78 (0.69–0.88)</td>
<td>&lt;.001</td>
<td>0.78 (0.69–0.88)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Platelets (cells/µL)</td>
<td>.999 (0.997–1)</td>
<td>.18</td>
<td>.998 (0.996–1)</td>
<td>.023</td>
<td></td>
</tr>
<tr>
<td>Platelets &lt;150 000 cells/µL</td>
<td>1.60 (1–2.54)</td>
<td>.049</td>
<td>1.92 (1.21–3.03)</td>
<td>.005</td>
<td></td>
</tr>
</tbody>
</table>

Ribotypes

014-020 | 0.73 (0.36–1.47) | .376 | 1.2 (0.63–2.31) | .576 |
027     | 1.96 (1.17–3.28)  | .01  | 2.68 (1.59–4.51) | <.001  |
053-163 | 0.91 (0.35–2.37)  | .851 | 1.65 (0.71–3.83) | .247  |
Ribotype 027c | 2.21 (1.35–3.62)  | .002 | 2.55 (1.57–4.13) | <.001  |
Positive toxin enzyme immunoassay | 1.38 (0.90–2.14) | .142 | 0.99 (0.63–1.55) | .974  |
Polymerase chain reaction cycle threshold (Ct) | 1.01 (0.93–1.09) | .873 | 1 (0.93–1.08) | .95  |

Abbreviations: CDI, *Clostridium difficile* infection; CI, confidence interval; OR, odds ratio.

* Missing values were imputed using a random-forest method.

As a categorical variable, referenced to other ribotypes.

As a binary variable.
mortality in hospitalized patients in a nonepidemic setting. The study also underscores concurrent use of antibiotics as an important, potentially modifiable risk factor for adverse outcomes. It demonstrates that serum bilirubin is an important component of the predictive model for both severe CDI and mortality; use of this predictor in models of outcomes from CDI has been previously little explored. This study does not support the hypotheses that CT or detection of stool toxins with EIA associates with severe CDI or mortality. Overall strengths of this study include the large size of the cohort; inclusion of both clinical and microbiological risk factors; and the agnostic, data-driven approach to modeling.

The association observed between infection with ribotype 027 and severe CDI is complex and was confounded by age; however, it did remain independently associated even after adjustment (OR, 1.73; 95% CI, 1.03–2.89; P = .037). Ribotype 027 was independently associated with mortality even after adjustment for a number of potential confounders, including age, comorbid disease, and PPI use (OR, 2.02; 95% CI, 1.19–3.43; P = .009). Ribotype 027 is known as an epidemic strain. Although our hospital’s rates of CDI are higher than national averages, they were stable during the study period; thus, our study was not conducted in an epidemic setting (data not publically available). Furthermore, data from our infection prevention program show no unit-specific

### Table 4. Multivariable Predictive Models for Severe Clostridium difficile Infection and 30-Day All-Cause Mortality

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Severe CDI</th>
<th>30-Day Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>1.02 (1.01–1.04)</td>
<td>.001</td>
</tr>
<tr>
<td>Charlson-Deyo score</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Prior CDI</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Prior CDI</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Solid organ neoplasm</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Metastatic cancer</td>
<td>2.60 (1.22–5.54)</td>
<td>.013</td>
</tr>
<tr>
<td>Liver disease</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>1.19 (0.66–2.12)</td>
<td>.566</td>
</tr>
<tr>
<td>Depression</td>
<td>1.65 (0.99–2.71)</td>
<td>.051</td>
</tr>
<tr>
<td>Concurrent antibiotic use</td>
<td>6.16 (2.8–13.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Proton pump inhibitor use</td>
<td>1.99 (1.11–3.6)</td>
<td>.022</td>
</tr>
<tr>
<td>Ribotype 027</td>
<td>1.73 (1.03–2.89)</td>
<td>.037</td>
</tr>
</tbody>
</table>

Variables potentially in the causal pathway between ribotype 027 infection and severe CDI or mortality were not considered for adjustment.

Abbreviations: CDI, Clostridium difficile infection; CI, confidence interval; OR, odds ratio.
outbreaks occurring during the study period, increasing the specificity of the association with adverse outcomes to PCR ribotype. Several possible explanations exist for why older adults are at increased risk of adverse outcomes from CDI. These include immunosenescence and decreased responsiveness to neoantigens, including toxins A and B [23]; decreased colonization resistance, with fewer competing anaerobes in the microbiome [24]; and greater exposure to risk factors for CDI, such as healthcare settings [25] and antimicrobials [26]. This study builds on evidence that infection with ribotype 027 is prevalent in older populations [10] but further suggests that it also plays an independent role in driving adverse outcomes. However, this association was not enough for entry into the predictive model for severe CDI (Table 4), suggesting that information about vitals and laboratory parameters may be more important for clinicians who wish to prognosticate about severity. In a previous study, our group failed to detect an independent association between ribotypes 027/078 and severe CDI in multivariate analysis. This study builds on evidence that infection with ribotype 027 is prevalent in older populations [10] but further suggests that it also plays an independent role in driving adverse outcomes. However, this association was not enough for entry into the predictive model for severe CDI (Table 4), suggesting that information about vitals and laboratory parameters may be more important for clinicians who wish to prognosticate about severity. In a previous study, our group failed to detect an independent association between ribotypes 027/078 and severe CDI in multivariate analysis. However, in that study, we combined the 2 ribotypes, did not independently examine mortality, and included only 310 cases of CDI in the derivation cohort [8]. A true independent contribution of ribotype 027 to disease outcome is important, as testing for this subtype of C. difficile is now available clinically (Xpert C. difficile/Epi; Cepheid, Sunnyvale, California) and the attributable mortality from infection with ribotype 027 could be significant, justifying implementation of preventative measures specific to this ribotype, such as fluoroquinolone restriction [27, 28] or more aggressive treatment.

In this study, we were unable to find an association between detectable toxin A or B in stool via EIA or Cₜ and either severe CDI or 30-day all-cause mortality, though overall detection of toxin by EIA was low at only 37.2%. Patients with ribotype 027 were more likely to have detectable stool toxin, but this phenotypic characteristic was not a contributor to outcomes. Furthermore, the analysis of Cₜ suggests that bacterial burden alone does not drive outcomes. The association between Cₜ and levels of stool toxin has also been evaluated in prior studies with conflicting results [29, 30]. The significance of detecting stool toxin A or B in relation to outcomes is unknown. In one of the larger studies to date, Planche et al [5] included 642 patients and demonstrated that detectable stool toxin by cell cytotoxicity assay was an independent, significant predictor of 30-day all-cause mortality. However, the minimal detectable toxin concentration by cell cytotoxicity assay is up to 3 orders of magnitude lower than by EIA [31], making it difficult to extrapolate their results to scenarios where other, less-sensitive tests are used.

This study found female gender, depression, and/or diabetes to be important variables in predictive models of severe CDI and mortality. Prior research has shown that population rates of CDI are higher in women than in men and that depression is associated with CDI [32]. Though human studies have reported...
sex differences in intestinal microbiota [33], including different relative abundances of Clostridia species [34], how gender-related differences in outcomes from CDI occur is presently undetermined. Diabetes was protective against mortality in the predictive model, and this paradoxical association has been previously described [35]. Further characterization of this relationship was beyond the scope of this study.

One of this study’s several potential limitations is that there were missing data. However, each relevant variable was closely examined, and the use of case-wise deletion or imputation was appropriately justified. Furthermore, the random-forest method used for imputation has evidence for superior performance in the setting of clinical prediction models [36]. Data were missing for $C_T$ in >15% of cases, and $C_T$ values were only available on the subset of patients who underwent tcdB gene testing (Figure 1). Thus, it was not possible to compare $C_T$ between patients with and without detectable toxin, as other studies have done [29, 30]. Additionally, all samples were collected at a single center that uses Cary-Blair media during transport. It is notable that only 37.2% of cases were diagnosed by detection of toxin in stool. The performance characteristics of toxin EIAs have been shown to vary considerably in the literature [37], but the sensitivity of the assay used could have affected the results. However, the overall sensitivity of the diagnostic algorithm (Figure 1) was >90%, even in Cary-Blair media, as demonstrated in a study conducted by our clinical laboratory [38]. Since we did not access the Social Security death file, it is also possible that we did not include some deaths due to incomplete follow-up. Finally, we used the McDonald et al [20] definition of severity, which is subjective since outcomes must be attributed to CDI. We attempted to ameliorate bias by using 2 independent reviewers for each case; however, it was not possible to mask the reviewers to patient characteristics. This limitation is common to all studies that use this composite definition of severity.

Conclusions
In summary, this is a novel study in its incorporation of detectable stool toxin by rapid testing, PCR CT, and PCR ribotype into an analysis of a large cohort alongside other predictors for severe CDI and all-cause mortality. It adds to the body of evidence that age, WBC, and/or infection with ribotype 027 play an important role in predictive models of adverse outcomes from CDI and demonstrates that ribotype 027 is independently associated with severe CDI. It also identifies concurrent antibiotic use as an important, potentially modifiable variable. However, it does not support the notion that $C_T$ or detectable stool toxin A or B by EIA are associated with adverse outcomes, though the low overall sensitivity of the toxin assay was notable. Further study is needed to help clarify the interaction between ribotype and other predictors such as age in relation to adverse events and to validate the importance of bilirubin in predictive models.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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