Changes in the Association Between Diagnostic Testing Method, Polymerase Chain Reaction Ribotype, and Clinical Outcomes From *Clostridioides difficile* Infection: One Institution's Experience

Anitha Menon,^{1,2,✉} D. Alex Perry,^{1,2} Jonathan Motyka,^{1,2} Shayna Weiner,^{1,2} Alexandra Standke,^{1,2} Aline Penkevich,^{1,2} Micah Keidan,^{1,2} Vincent B. Young,^{1,2,3} and Krishna Rao^{1,2}

¹Department of Internal Medicine, Division of Infectious Diseases, University of Michigan, Ann Arbor, Michigan, USA, ²University of Michigan Medical School, Ann Arbor, Michigan, USA, and

³Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan, USA

Background. In *Clostridioides difficile* infection (CDI), the relationship between clinical, microbial, and temporal/epidemiological trends, disease severity and adverse outcomes is incompletely understood. In a follow-up to our study from 2010–2013, we evaluate stool toxin levels and *C. difficile* polymerase chain reaction (PCR) ribotypes. We hypothesized that elevated stool toxins and infection with ribotype 027 associate with adverse outcomes.

Methods. In 565 subjects at the University of Michigan with CDI diagnosed by positive testing for toxins A/B by enzyme immunoassay (EIA) or PCR for the *tcdB* gene, we quantified stool toxin levels via a modified cell cytotoxicity assay (CCA), isolated *C. difficile* by anaerobic culture, and performed PCR ribotyping. Severe CDI was defined by Infectious Diseases Society of America (IDSA) criteria, and primary outcomes were all-cause 30-day mortality and a composite of colectomy, intensive care unit admission, and/or death attributable to CDI within 30 days. Analyses included bivariable tests and logistic regression.

Results. 199 samples were diagnosed by EIA; 447 were diagnosed by PCR. Toxin positivity associated with IDSA severity but not primary outcomes. In 2016, compared with 2010–2013, ribotype 106 newly emerged, accounting for 10.6% of strains, ribotype 027 fell from 16.5% to 9.3%, and ribotype 014–027 remained stable at 18.9%. Ribotype 014–020 associated with IDSA severity and 30-day mortality ($P = .001$).

Conclusions. Toxin positivity by EIA and CCA associated with IDSA severity but not with subsequent adverse outcomes. The molecular epidemiology of *C. difficile* has shifted, which may have implications for the optimal diagnostic strategy for and clinical severity of CDI.

Keywords. *C. difficile*; diagnostic testing methods; toxins; infection prevention; molecular epidemiology.

Clostridioides difficile infection (CDI) is the most common cause of infectious nosocomial diarrhea in the United States [1]. *Clostridioides difficile* infection leads to a spectrum of clinical syndromes, ranging from self-limited diarrheal illness to fulminant colitis [2]. The morbidity and mortality due to CDI have increased significantly in the decade from 2000 to 2010, and *C. difficile* is now responsible for over 450 000 cases of infection and nearly 30 000 deaths per year in the United States alone [3]. This epidemic was thought to be driven by the emergence of a new variant of *C. difficile* variously referred to as ribotype 027/REA type BI/PFGE type NAP1 (hereafter referred to as ribotype

027). However, whether ribotype 027 is hypervirulent is controversial due to its strong epidemiological link to age, which is independently associated with mortality [4–6]. Furthermore, the epidemiology of CDI has changed in the subsequent decade, and it is not well established that ribotype 027 is still associated with severe disease and adverse outcomes in a nonendemic setting.

The optimal diagnostic testing method for CDI remains controversial. No test clearly differentiates colonization from symptomatic infection, and diagnosis of CDI ultimately relies on a combination of laboratory testing and clinical suspicion [7]. The pathogenesis of CDI is mediated by toxins A and/or B, and there is evidence that the amount of toxin production varies from strain to strain [8]. In addition to the strain, the role of detectable stool toxin in prognosis may be considered. Rapid tests for diagnosis usually detect toxins A and/or B directly in stool by enzyme immunoassay (EIA) or the gene for toxin B, *tcdB*, by real-time polymerase chain reaction (PCR). It has been argued that a PCR-only approach for diagnosing CDI,

Received 3 June 2020; editorial decision 3 September 2020; published online 15 September 2020.

Correspondence: K. Rao, University of Michigan, 1150 W. Medical Center Dr., MSRB1 1510B, Ann Arbor, MI 48103 (krirao@med.umich.edu).

Clinical Infectious Diseases® 2021;73(9):e2883–9

© The Author(s) 2020. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.

DOI: 10.1093/cid/ciaa1395

while more sensitive, is not optimal as PCR may be positive in colonized patients without disease. Some studies have found an association between detection of toxin in the stool, rather than the organism alone, and adverse outcomes such as all-cause mortality [9].

Here, we attempt to deconvolute the relationships between clinical variables, ribotype, stool toxin levels, severe disease, and adverse outcomes in patients with CDI. Our group previously found an association between ribotype 027 and adverse outcomes [10]. We found no association between detectable toxin A/B in the stool via EIA and adverse outcomes, but the use of Cary-Blair media in this study may have led to preanalytic dilution and false-negative EIA results, and our institution no longer follows this practice [10, 11]. Moreover, strains differ with respect to toxin production, and the molecular epidemiology of *C. difficile* has since changed at our institution. Thus, we felt it important to revisit these relationships in a new cohort. This study has 2 main objectives: (1) to assess the associations between stool toxin, disease severity, and adverse outcomes in patients with CDI and (2) to assess the distribution of *C. difficile* ribotypes in patients with CDI at our institution and the relationship between ribotype and adverse outcomes. We hypothesized that stool toxin A/B positivity and infection with ribotype 027 would associate with severe disease and adverse outcomes.

METHODS

Study Design and Patients

The University of Michigan (UM) Institutional Review Board approved this cohort study with prospective sample collection and retrospective data abstraction of nonpregnant hospitalized patients of aged 18 years or older from the University of Michigan between February and December 2016. We consecutively evaluated for inclusion any unformed stool samples that were submitted to the microbiology laboratory for *C. difficile* testing. After rejection of formed specimens, all included samples tested positive for the presence of toxigenic *C. difficile* by the clinical microbiology laboratory's 2-step testing protocol (see below). All laboratory testing of inpatients was performed at the discretion of the inpatient team, who ordered *C. difficile* testing per institutional guidelines that recommend testing of symptomatic patients with suspected CDI [12]. We excluded positive samples from the same patient within 2 weeks of the index sample, as they were not thought to represent separate episodes of CDI.

Microbiology

Clostridioides difficile infection cases were identified using a tiered approach in which clinical specimens submitted for *C. difficile* testing were first processed using a combined glutamate dehydrogenase antigen EIA and toxin A/B EIA (C. Diff Quik Chek Complete; Alere, Kansas City, MO). In instances of

concordance, the results are reported as negative or positive. In instances of discordance, reflex testing by PCR for presence of toxin B gene (*tcdB*) using a commercial assay was conducted. At UM, the GeneOhm assay (Becton Dickinson, Franklin Lakes, NJ) was used through 2013 and the Simplexa assay (Focus Diagnostics, Cypress, CA) was used thereafter. On all samples, we quantified stool toxin activity levels via a modified cell cytotoxicity assay (CCA) (Supplementary Material Methods). We also cultivated *C. difficile* by anaerobic culture, confirmed isolates to be toxigenic using the multiplex PCR developed by Persson et al, and performed PCR ribotyping, as previously described [13, 14].

Data Extraction

We extracted patient data on demographics, comorbid disease (taken from International Classification of Disease, 10th revision [ICD-10], codes), vitals, laboratory test results, medications, and outcomes from the electronic medical record through structured query. We defined high-risk antibiotics as those with broad-spectrum activity and demonstrated associations with risk of CDI (third- or fourth-generation cephalosporins, fluoroquinolones, lincosamides, β -lactam/ β -lactamase inhibitor combinations, oral vancomycin, and carbapenems), as previously described by Baggs et al [15]. We included values for vitals and laboratory results if available within 24–48 hours of diagnosis and calculated unweighted Charlson-Deyo comorbidity scores from ICD-10 codes. Infectious Diseases Society of America (IDSA) severity was defined as follows: white blood cell (WBC) count more than 15 000 cells/ μ L or a 1.5-fold increase in serum creatinine above baseline [16]. One of our primary outcomes, 30-day all-cause mortality, while unambiguous and extractable from the chart through automated query, is a heterogeneous outcome not necessarily due to CDI. Thus, we also assessed for associations with disease-related complications (DRCs), defined as occurrence of any of the following outcomes attributed to CDI within 30 days of diagnosis: admission to an intensive care unit, colectomy, or death. Clinicians from the study team reviewed outcomes and determined whether they were attributable to CDI.

Data Analyses

All analyses were conducted in R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) and a 2-tailed *P* value less than .05 was considered statistically significant. The primary outcomes were all-cause 30-day mortality and DRCs as defined above.

To test the hypothesis that stool toxin (either by the clinical EIA or by CCA) associates with DRCs and mortality, we assessed bivariable relationships between toxin positivity, all potential covariates, and outcomes using logistic regression. Next, we developed multivariable logistic regression models including variables not expected to be in the causal pathway

between toxin production and adverse outcomes (eg, demographics, comorbid disease, and certain laboratory tests), using the conceptual diagram shown in Figure 1. Finally, we conducted a subgroup analysis on inpatients with CDI, as this group is more likely to have adverse outcomes from CDI, and a sensitivity analysis by excluding IDSA severity from the final parsimonious models (Supplementary Material Table S1).

To assess epidemiological trends in ribotype strain at our institution, we compared the period prevalence of the most prevalent ribotypes as defined above from our 2016 cohort to a cohort from 2010–2013, on which we previously published [10]. We then conducted 2-proportion z-tests to determine whether the period prevalence of ribotypes changed between the 2 periods.

RESULTS

Similarity of Baseline Characteristics of This Cohort to Our 2010–2013 Cohort

Samples from 647 discrete episodes of CDI from 565 adult patients met study criteria for inclusion. Selected study population characteristics are summarized in Table 1; 199 (30.8%) were diagnosed by a positive toxin A or B EIA test and the remainder (448) by a positive by reflex PCR for *tcdB*. A total of 411 cases (76.1%) had toxin A/B levels greater than 6.4 ng/mL by CCA; of these, 166 were positive by EIA and 245 were positive by PCR. Older adults (aged >65 years) comprised 38.5% of cases. Concurrent non-CDI antibiotic use occurred in 52

cases (14.6%); 63 distinct ribotypes were identified, but there were more isolates of ribotypes 014–020, 027, 106, and 002 than any other ribotype. There were 142 (35.7%) meeting criteria for IDSA severity, 40 (6.2%) with 30-day all-cause mortality, and 50 (7.7%) with DRCs.

Initial Bivariable Relationships Reveal a Complex Relationship Among Stool Toxin, Ribotype, Comorbidities, and Outcomes

Figure 1 shows the causal framework we used when approaching the analyses for this study, with toxin production central to the pathogenesis of CDI. To identify the potential confounders to include in our initial multivariable models, we assessed for associations with the 2 toxin assays and noted the following at $P < .1$: detectable stool toxin by EIA was associated with age, inflammatory bowel disease, albumin trough, total bilirubin peak, ribotype 027, and ribotype 014–020. Our other toxin assay variable, quantitative toxin greater than 6.4 ng/mL, was associated with female sex, WBC peak, fever, and ribotype 027. Detectable stool toxin by EIA and quantitative toxin greater than 6.4 ng/mL were also associated with each other (odds ratio [OR], 86.7; $P < .001$).

Next, we examined how the toxin assay variables related to IDSA severity and our primary outcomes, 30-day all-cause mortality and DRCs. Detectable stool toxin by EIA and quantitative toxin greater than 6.4 ng/mL were associated with IDSA severity (OR, 1.87 [$P = .006$] and 2.38 [$P = .006$], respectively),

Figure 1. Proposed causal diagram of CDI and adverse outcomes. This framework purports that toxin production following infection by *Clostridioides difficile* is central to the pathogenesis and development of IDSA severity (white blood cell count >15 000 cells/ μ L or a 1.5-fold increase in serum creatinine above baseline). This, in turn, leads to increased risk of the other outcomes, 30-day all-cause mortality, and DRCs (defined as occurrence of any of the following outcomes attributed to CDI within 30 days of diagnosis: admission to an intensive care unit, colectomy, or death). This risk is, in turn, potentially modulated by confounders and/or other unmeasured factors (gray lines). Abbreviations: CCA, cell cytotoxicity assay; CDI, *Clostridioides difficile* infection; DRC, disease-related complication; EIA, enzyme immunoassay; IDSA, Infectious Diseases Society of America; OR, odds ratio.

Table 1. Selected Characteristics and Outcomes in the Study Population of 647 Cases of *Clostridioides difficile* Infection

Variable	n (%) or Mean $\pm$ SD (95% CI)	No. Included ^a
Age, years	57.7 $\pm$ 18.2	647
Female sex	344 (53.2)	647
White race	559 (86.9)	643
Location		647
Inpatient	429 (66.3)	
Outpatient	148 (22.9)	
ED	38 (5.9)	
Other (eg, nursing home)	32 (5.0)	
Elixhauser score	4.09 $\pm$ 3.23	421
Meets IDSA severity criteria	142 (35.7)	398
Prior CDI episodes	0.63 $\pm$ 1.33	647
IBD	109 (16.8)	647
High-risk prior antibiotic use	25 (7.0)	357
High-risk concurrent antibiotic use	52 (14.6)	357
Fever (>38°C)	156 (31.1)	501
Systolic blood pressure, mm Hg	93.9 $\pm$ 18.6	503
Maximal respiratory rate, breaths/minute	31.4 $\pm$ 21.8	480
Ileus	43 (6.7)	647
Megacolon	1 (0.2)	647
White blood cell count, cells/ μ L	12.2 $\pm$ 15.5	550
Sodium, mg/dL	138 $\pm$ 3.78	546
Creatinine, mg/dL	1.30 $\pm$ 1.42	546
Acute kidney injury	27 (7.8)	346
Albumin, g/dL	3.30 $\pm$ 0.74	442
Total bilirubin, mg/dL	1.22 $\pm$ 2.53	424
Hemoglobin, g/dL	10.0 $\pm$ 2.35	550
Platelets, 1000 cells/ μ L	259 $\pm$ 135	550
C-reactive protein, mg/dL	5.69 $\pm$ 6.43	94
Detectable stool toxin by EIA	199 (30.8)	647
Quantitative toxin >6.4 ng/mL ^b	411 (76)	540
30-Day all-cause mortality	40 (6.2)	647
DRCs ^c	50 (7.7)	647
Ribotypes, n		558
027	52	...
014–020	105	...
106	599	...
002	53	...

Abbreviations: CDI, *Clostridioides difficile* infection; DRC, disease-related complication; ED, emergency department; EIA, enzyme immunoassay; IBD, inflammatory bowel disease; IDSA, Infectious Diseases Society of America.

^aNumber of patients with available data on this variable.

^bMeasured by a custom cell cytotoxicity assay.

^cDefined as occurrence of any of the following outcomes attributed to CDI within 30 days of diagnosis: admission to an intensive care unit, colectomy, or death.

but not with the primary outcomes (Table 2, Figure 1). IDSA severity was associated with both 30-day mortality (OR, 9.34; $P \leq .001$) and DRCs (OR, 5.16; $P \leq .001$).

Stool Toxin Is Not Associated With Adverse Outcomes From *Clostridioides difficile* Infection

Even though both detectable stool toxin by EIA and quantitative toxin greater than 6.4 ng/mL associated with IDSA severity, which, in turn, associated with both of the primary outcomes

(Figure 1), neither toxin variable was associated with our primary outcomes on unadjusted analysis. Thus, based on this and a priori evidence from the literature, we suspected the relationship to primary outcomes was confounded or modulated by certain markers of disease severity such as age or end-organ damage, and we adjusted for those putative confounders to assess if a masked association between toxin assays and primary outcomes was present. After adjustment for putative confounders and effect modifiers (see section above), we still did not observe an association between either toxin variable and our primary outcomes (data not shown). The only significant interaction term we noted was between toxin by EIA and age in the model for DRCs ($\beta = -9.39$; $P = .017$ for interaction term in model). Although the interaction term was significant, when included in the model, age became nonsignificant, and all confidence intervals for the ORs of DRCs versus toxin detection at various ages crossed unity (data not shown). This indicates that the interaction did not meaningfully contribute to our analysis of toxin by EIA versus DRCs, and we excluded it. Final multivariable models are presented in Tables 3 and 4, and neither toxin variable was significantly associated with either primary outcome in these models.

As IDSA severity is measured by WBC and creatinine, elevations of which are often associated with infection, this variable was suspected to be in the causal pathway between infection and DRCs (Figure 1). As this could have been diluting true relationships between toxins and outcomes, we conducted sensitivity analyses by excluding IDSA severity from the final parsimonious models, where applicable, and still did not observe an association between toxin positivity and our primary outcomes. Although not observed in our own cohort (Table 2), since an association between ribotype 027 and CDI severity has previously been described, we also included ribotype 027 in our final models, yet still did not observe a significant association between toxin positivity and our primary outcomes (data not shown). Finally, when we restricted our sample to inpatients as a subgroup analysis, we still did not observe any unadjusted or adjusted associations between toxin positivity and outcomes (Supplementary Table S1).

The Molecular Epidemiology of *Clostridioides difficile* Has Shifted Over Time at Our Institution

In our analysis of *C. difficile* strain by ribotype, we found that ribotype 014–020 was associated with both 30-day mortality ($P = .001$) and DRCs ($P = .01$) (Table 2). Ribotype 027 was notably not associated with these clinical outcomes. In trying to understand this phenomenon, we found that, compared with the period from 2010–2013, the circulating ribotypes of *C. difficile* at our institution changed in 2016 (Table 5). Notably, the prevalence of ribotype 106 increased to 10.6% and ribotype 027 decreased to 9.3%. The prevalence of ribotype 014–020 remained stable at 18.9%.

Table 2. Variables Versus Severity and Outcomes (Unadjusted)

Variable	IDSA Severity		30-Day Mortality		DRCs ^a	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age	1.00 [0.99, 1.01]	.745	1.03 [1.01, 1.05]	.009**	1.03 [1.01, 1.06]	.013*
White race	1.33 [0.71, 2.60]	.384	0.82 [0.35, 2.22]	.658	0.40 [0.65, 0.26]	.394
Female sex	1.05 [0.69, 1.58]	.823	1.27 [0.67, 2.44]	.459	1.05 [0.69, 1.58]	.823
Albumin, g/dL	0.58 [0.41, 0.80]	.001***	0.14 [0.07, 0.26]	<.001***	0.20 [0.10, 0.37]	<.001***
Total bilirubin, mg/dL	1.12 [1.02, 1.26]	.028*	1.25 [1.13, 1.40]	<.001***	1.28 [1.16, 1.44]	<.001***
PPI	1.84 [1.07, 3.21]	.030*	2.38 [1.02, 6.52]	.063	5.01 [1.42, 31.8]	.032*
Prior high-risk antibiotic	1.03 [0.39, 2.53]	.957	2.71 [0.85, 7.32]	.063	1.43 [0.22, 5.36]	.642
Concurrent high-risk antibiotic	1.42 [0.73, 2.71]	.295	0.79 [0.23, 2.13]	.677	1.92 [0.61, 5.17]	.223
IBD	0.75 [0.42, 1.30]	.318	0.12 [0.01, 0.55]	.036*	0.58 [0.14, 1.69]	.381
Ribotypes						
027	1.57 [0.69, 3.51]	.275	0.26 [0.01, 1.59]	.238	0.40	.719
014–020	1.21 [0.76, 2.29]	.320	3.33 [1.59, 6.80]	.001	3.17 [1.28, 7.57]	.010*
106	0.72 [0.33, 1.48]	.387	0.51 [0.08, 1.75]	.367	0.84 [0.13, 2.98]	.818
002	1.63 [0.78, 3.40]	.190	0.28 [0.02, 1.32]	.208	0.39 [0.01, 2.50]	.498
Detectable stool toxin by EIA	1.85 [1.18, 2.89]	.007**	1.09 [0.53, 2.12]	.805	0.74 [0.29, 1.69]	.501
Quantitative toxin >6.4 ng/mL ^b	2.11 [1.22, 3.80]	.009**	1.44 [0.52, 3.93]	.430	0.83 [0.33, 2.36]	.704

Significance levels: * $P < .05$, ** $P < .01$, *** $P < .001$.

Abbreviations: CDI, *Clostridioides difficile* infection; DRC, disease-related complication; EIA, enzyme immunoassay; IBD, inflammatory bowel disease; IDSA, Infectious Diseases Society of America; OR, odds ratio; PPI, proton pump inhibitors.

^aDefined as occurrence of any of the following outcomes attributed to CDI within 30 days of diagnosis: admission to an intensive care unit, colectomy, or death.

^bMeasured by a custom cell cytotoxicity assay.

DISCUSSION

This study did not identify any association between detectable stool toxin by EIA or CCA and adverse clinical outcomes. This finding held after accounting for possible confounding/effect modification and across important subgroups/sensitivity analyses. This study also identified epidemiological shifts in *C. difficile* ribotypes at our institution, with the period prevalences of ribotypes 106 and 014–020 surpassing that of ribotype 027 in 2016 when compared with 2010–2013. Alongside this shift in molecular epidemiology, our findings regarding ribotype 027 and adverse outcomes were discordant with prior studies [17, 18]. In our cohort study from 2010–2013, we identified ribotype 027 as being independently

associated with DRCs, but here we were unable to replicate this finding [10].

We saw an association between toxin levels and IDSA severity, as well as an association between IDSA severity and outcomes. Because we did not find an association between toxin levels and outcomes, it appears that, while IDSA severity—a composite of peripheral WBC count and creatinine—may play a role in the causal pathway from toxin to outcomes, factors other than stool toxin levels may be necessary or more important in increasing the risk of adverse outcomes. For example, since toxin detected in stool, by definition, did not bind to receptors and cause an inflammatory response, incorporating both toxin levels and measures of the inflammatory response through stool biomarkers such as interleukin-8 may be a promising approach for future studies [19]. Our result is also congruous with a prior

Table 3. Adjusted Analysis of Detectable Stool Toxin by Enzyme Immunoassay Versus 30-Day All-cause Mortality and Disease-related Complications

Predictor	30-Day Mortality		DRCs ^a	
	OR (95% CI)	P	OR (95% CI)	P
Toxin positive by EIA	1.14 [0.40, 3.07]	.80	0.68 [0.25, 1.68]	.413
Age, years	1.04 [1.01, 1.09]	.02*	1.03 [1.00, 1.07]	.024*
Albumin, g/dL	0.26 [0.11, 0.56]	.001**	0.20 [0.10, 0.40]	<.001***
Total bilirubin, mg/dL	4.86 [1.82, 13.80]	.002**	...	
IDSA severity	7.37 [2.70, 23.05]	<.001***	...	

Significance levels: * $P < .05$, ** $P < .01$, *** $P < .001$.

Abbreviations: CDI, *Clostridioides difficile* infection; DRC, disease-related complication; EIA, enzyme immunoassay; IDSA, Infectious Diseases Society of America; OR, odds ratio.

^aDefined as occurrence of any of the following outcomes attributed to CDI within 30 days of diagnosis: admission to an intensive care unit, colectomy, or death

Table 4. Adjusted Analysis of Quantitative Toxin >6.4 ng/mL Versus 30-Day All-cause Mortality and Disease-related Complications

Predictor	30-Day Mortality		DRCs ^a	
	OR (95% CI)	P	OR (95% CI)	P
Toxin positive by CCA >6.4	1.23 [0.42, 4.44]	.731	0.65 [0.24, 2.02]	.80
IDSA severity	10.35 [3.79, 36.41]	<.001***	7.69 [2.67, 27.92]	<.001***

Significance level: *** $P < .001$.

Abbreviations: CCA, cell cytotoxicity assay; CDI, *Clostridioides difficile* infection; DRC, disease-related complication; IDSA, Infectious Diseases Society of America; OR, odds ratio.

^aDefined as occurrence of any of the following outcomes attributed to CDI within 30 days of diagnosis: admission to an intensive care unit, colectomy, or death.

Table 5. Period Prevalence of Most Common *Clostridioides difficile* Ribotypes in the Study (2016 vs 2010–2013)

Ribotype	2010–2013, n (%)	2016, n (%)	P
Total	1099	557	
027	181 (16.5)	52 (9.3)	<.001***
014–020	178 (16.2)	105 (18.9)	.198
053–163	72 (6.6)	15 (2.7)	.001***
078–126	33 (3.0)	9 (1.6)	.126
106	0 (0)	59 (10.6)	<.001***

Data from reference 10. Significance level: *** $P < .001$.

large, multicenter study that did not find an association between toxin detection by EIA and severe outcomes [20].

Our findings of no association between stool toxin positivity and 30-day mortality are analogous to those in our 2015 study [10]. A limitation of this previous paper was that samples had been collected in Cary-Blair media, which could potentially have led to toxin dilution. Our current study was done with stool samples not collected in media. Moreover, we conducted cytotoxicity assays of high analytic sensitivity to quantify the actual amount of toxin present in samples, which still did not correlate with outcomes.

The literature shows conflicting evidence of the association between stool toxin positivity and adverse clinical outcomes. Some studies that demonstrated this link did not report *C. difficile* strain information. Since strains can differ in the severity of disease they cause, it is difficult to compare our study with studies that do not report strain information. This is also true since strains responsible for infection differ over time and among geographically distant sites [6, 17, 21–24]. Other studies that demonstrated this link were conducted in cohorts that had a high prevalence of ribotype 027, or were conducted during the ribotype 027 epidemic era (2000–2010) [25, 26]. These factors could have driven part of the association, as ribotype 027 has previously been associated with both high toxin production and clinical severity [17, 21, 27]. Given the confounding effect of ribotype, toxin levels may not be the most important factor when predicting clinical outcomes [28]. Most studies that did not find an association between toxin levels and adverse clinical outcomes had samples with low ribotype 027 prevalence, comparable to our study (10–20%) [29, 30].

Our data also demonstrate an association between ribotype 014–020 and adverse outcomes, which we did not previously observe. It is beyond the scope of this study to explain why this association exists. Some possible explanations include sampling artifact—the shift in ribotypes leading to 014–020 being the predominant one (Table 5)—leading to a proportionately higher frequency of this ribotype among severe cases of CDI. Further studies should focus on this specific finding to confirm or refute it.

That we did not see an association between ribotype 027 and outcomes could also be partially due to epidemiological

shifts in *C. difficile* at our institution, particularly the emergence of ribotype 106 and increase in ribotype 014–020. This raises several hypotheses that should be further studied. First, some ribotype 106 isolates also harbor fluoroquinolone resistance, and could therefore be outcompeting ribotype 027. Second, the reduced prevalence of ribotype 027 could be diluting the association between this ribotype and severity due to low statistical power. Third, since we know that strains such as ribotype 027 can have widely variant phenotypes, it is also possible that the endemic variants of ribotype 027 that persist in the presence of ribotype 106 are less virulent, negating the association between ribotype and outcomes [8].

Our study has several limitations. First, this is a retrospective study, so not all relevant data were available for collection. For instance, we were unable to differentiate true cases of *C. difficile* from colonization with absolute certainty, relying on documentation in clinical records. Although all patients included in this study had diarrhea, were clinically suspected to have CDI by the treating physicians, and no formed stools were accepted by the laboratory, it was not possible to confirm whether all patients met the IDSA criteria for CDI through retrospective chart review (>3 episodes of unformed stool in 24 hours without an alternate explanation), due to limitations of such documentation in our electronic health records [12]. Next, the overall cohort size is smaller than in our prior study and some other published studies, which decreases our power to detect small effect sizes. However, we were able to detect ORs as low as 2 (for binary variables).

Future work should attempt to identify whether an association exists between toxin by EIA or CCA and other outcomes, such as duration of symptoms, time to resolution of diarrhea, and time to clinical cure. This may require a prospective study, as these variables are difficult or even impossible to extract retrospectively, depending on the institution. If toxin detection associates with these other outcomes, this could affect duration of hospitalization, and incorporation of such a metric could facilitate hospital systems' efforts to both increase patient safety and reduce healthcare overutilization. Research efforts should also focus on whether toxin quantification is able to distinguish colonization from true CDI. Incorporation of stool biomarkers alongside toxin levels may help with these efforts [19].

CONCLUSIONS

Toxin detection by EIA or CCA associated with case severity as measured by the IDSA criteria, but this study was unable to confirm an association with subsequent adverse outcomes. The molecular epidemiology of *C. difficile* varies over time and geography, and this may have implications for the optimal diagnostic strategy for CDI at specific institutions. It remains to be seen whether there is prognostic clinical utility with toxin assays in CDI.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. Parts of this work were presented at IDWeek 2019 in Washington, DC.

Disclaimer. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Financial support. This work was supported by grants from the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (grant numbers U19AI090871, R21AI120599, and U01AI124255).

Potential conflicts of interest. K. R. has consulted for Bio-K+ International, Inc and Roche Molecular Systems, Inc. V. B. Y. has consulted for Bio-K+ International, Inc, Pantheryx, Exarcia Pharmaceuticals, and Vedanta. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

- Polage CR, Solnick JV, Cohen SH. Nosocomial diarrhea: evaluation and treatment of causes other than *Clostridium difficile*. Clin Infect Dis 2012; 55:982–9.
- Hurley BW, Nguyen CC. The spectrum of pseudomembranous enterocolitis and antibiotic-associated diarrhea. Arch Intern Med 2002; 162:2177–84.
- Lessa FC, Mu Y, Bamberg WM, et al. Burden of *Clostridium difficile* infection in the United States. N Engl J Med 2015; 372:825–34.
- Louie TJ, Miller MA, Crook DW, et al. Effect of age on treatment outcomes in *Clostridium difficile* infection. J Am Geriatr Soc 2013; 61:222–30.
- Yakob L, Riley TV, Paterson DL, et al. Mechanisms of hypervirulent *Clostridium difficile* ribotype 027 displacement of endemic strains: an epidemiological model. Sci Rep 2015; 5:12666.
- Gerding DN. Global epidemiology of *Clostridium difficile* infection in 2010. Infect Control Hosp Epidemiol 2010; 31:S32–4.
- Bagdasarian N, Rao K, Malani PN. Diagnosis and treatment of *Clostridium difficile* in adults: a systematic review. JAMA 2015; 313:398–408.
- Carlson PE Jr, Kaiser AM, McColm SA, et al. Variation in germination of *Clostridium difficile* clinical isolates correlates to disease severity. Anaerobe 2015; 33:64–70.
- Kraft CS, Parrott JS, Cornish NE, et al. A laboratory medicine best practices systematic review and meta-analysis of nucleic acid amplification tests (NAATs) and algorithms including NAATs for the diagnosis of Clostridioides *Clostridium difficile* in adults. Clin Microbiol Rev 2019; 32:e00032–18.
- Rao K, Micic D, Natarajan M, et al. *Clostridium difficile* ribotype 027: relationship to age, detectability of toxins A or B in stool with rapid testing, severe infection, and mortality. Clin Infect Dis 2015; 61:233–41.
- Fang FC, Polage CR, Wilcox MH. Point-counterpoint: what is the optimal approach for detection of *Clostridium difficile* J Clin Microbiol 2017; 55:670–80.
- Cohen SH, Gerding DN, Johnson S, et al; Society for Healthcare Epidemiology of America; Infectious Diseases Society of America. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol 2010; 31:431–55.
- Martinson JN, Broadway S, Lohman E, et al. Evaluation of portability and cost of a fluorescent PCR ribotyping protocol for *Clostridium difficile* epidemiology. J Clin Microbiol 2015; 53:1192–7.
- Persson S, Torpdahl M, Olsen KE. New multiplex PCR method for the detection of *Clostridium difficile* toxin A (tcdA) and toxin B (tcdB) and the binary toxin (cdtA/cdtB) genes applied to a Danish strain collection. Clin Microbiol Infect 2008; 14:1057–64.
- Baggs J, Jernigan JA, Halpin AL, Epstein L, Hatfield KM, McDonald LC. Risk of subsequent sepsis within 90 days after a hospital stay by type of antibiotic exposure. Clin Infect Dis 2018; 66:1004–12.
- McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis 2018; 66:e1–48.
- Planche TD, Davies KA, Coen PG, et al. Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnostic validation study of C difficile infection. Lancet Infect Dis 2013; 13:936–45.
- See I, Mu Y, Cohen J, et al. NAP1 strain type predicts outcomes from *Clostridium difficile* infection. Clin Infect Dis 2014; 58:1394–400.
- Steiner TS, Flores CA, Pizarro TT, Guerrant RL. Fecal lactoferrin, interleukin-1beta, and interleukin-8 are elevated in patients with severe *Clostridium difficile* colitis. Clin Diagn Lab Immunol 1997; 4:719–22.
- Guh AY, Hatfield KM, Winston LG, et al. Toxin enzyme immunoassays detect clostridioides difficile infection with greater severity and higher recurrence rates. Clin Infect Dis 2019; 69:1667–74.
- Polage CR, Gyorke CE, Kennedy MA, et al. Overdiagnosis of *Clostridium difficile* infection in the molecular test era. JAMA Intern Med 2015; 175:1792–801.
- Patel H, Randhawa J, Nanavati S, Marton LR, Baddoura WJ, DeBari VA. Laboratory and clinical features of EIA toxin-positive and EIA toxin-negative community-acquired *Clostridium difficile* infection. Ann Clin Lab Sci 2015; 45:333–9.
- Kumar S, Pollok R, Muscat I, Planche T. Diagnosis and outcome of *Clostridium difficile* infection by toxin enzyme immunoassay and polymerase chain reaction in an island population. J Gastroenterol Hepatol 2017; 32:415–9.
- Yuhashi K, Yagihara Y, Misawa Y, et al. Diagnosing *Clostridium difficile*-associated diarrhea using enzyme immunoassay: the clinical significance of toxin negativity in glutamate dehydrogenase-positive patients. Infect Drug Resist 2016; 9:93–9.
- Beaulieu C, Dionne LL, Julien AS, Longtin Y. Clinical characteristics and outcome of patients with *Clostridium difficile* infection diagnosed by PCR versus a three-step algorithm. Clin Microbiol Infect 2014; 20:1067–73.
- Guerrero DM, Chou C, Jury LA, Nerandzic MM, Cadnum JC, Donskey CJ. Clinical and infection control implications of *Clostridium difficile* infection with negative enzyme immunoassay for toxin. Clin Infect Dis 2011; 53:287–90.
- Pant C, Sfera TJ, Deshpande A, Minocha A. Clinical approach to severe *Clostridium difficile* infection: update for the hospital practitioner. Eur J Intern Med 2011; 22:561–8.
- Carlson PE Jr, Walk ST, Bourgis AE, et al. The relationship between phenotype, ribotype, and clinical disease in human *Clostridium difficile* isolates. Anaerobe 2013; 24:109–16.
- Berry N, Sewell B, Jafri S, et al. Real-time polymerase chain reaction correlates well with clinical diagnosis of *Clostridium difficile* infection. J Hosp Infect 2014; 87:109–14.
- Kaltsas A, Simon M, Unruh LH, et al. Clinical and laboratory characteristics of *Clostridium difficile* infection in patients with discordant diagnostic test results. J Clin Microbiol 2012; 50:1303–7.