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Toxin Enzyme Immunoassays Detect *Clostridioides difficile* Infection With Greater Severity and Higher Recurrence Rates

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Abstract

Background—Few data suggest that *Clostridioides difficile* infections (CDIs) detected by toxin enzyme immunoassay (EIA) are more severe and have worse outcomes than those detected by nucleic acid amplification tests (NAATs) only. We compared toxin-positive and NAAT-positive-only CDI across geographically diverse sites.

Methods—A case was defined as a positive *C. difficile* test in a person ≥1 year old with no positive tests in the prior 8 weeks. Cases were detected during 2014–2015 by a testing algorithm (specimens initially tested by glutamate dehydrogenase and toxin EIA; if discordant results, specimens were reflexed to NAAT) and classified as toxin positive or NAAT positive only. Medical charts were reviewed. Multivariable logistic regression models were used to compare CDI-related complications, recurrence, and 30-day mortality between the 2 groups.

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Potential conflicts of interest. D. N. G. is a member of the scientific advisory boards of Merck, Rebiotix, Actelion, DaVolterra, and Summit; is a consultant for Pfizer, MGB Pharma, and Sanofi Pasteur; holds a research grant from Seres Therapeutics; and holds patents and technology for the prevention of *Clostridioides difficile* infection. G. D. serves on the drug safety monitoring board for a *C. difficile* treatment study by Seres Therapeutics. M. K. serves on the Board of Directors of the Infectious Disease Consulting Corporation; has received conference travel reimbursement from the Society for Healthcare Epidemiology of America, the Council of State and Territorial Epidemiologists, and the Association for Professionals in Infection Control and Epidemiology; and reports an honorarium and travel reimbursement from Medscape. 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.

Results—Of 4878 cases, 2160 (44.3%) were toxin positive and 2718 (55.7%) were NAAT positive only. More toxin-positive than NAAT-positive-only cases were aged ≥65 years (48.2% vs 38.0%; $P < .0001$), had ≥3 unformed stools for ≥1 day (43.9% vs 36.6%; $P < .0001$), and had white blood cell counts ≥15 000 cells/μL (31.4% vs 21.4%; $P < .0001$). In multivariable analysis, toxin positivity was associated with recurrence (adjusted odds ratio [aOR], 1.89; 95% confidence interval [CI], 1.61–2.23), but not with CDI-related complications (aOR, 0.91; 95% CI, .67–1.23) or 30-day mortality (aOR, 0.95; 95% CI, .73–1.24).

Conclusions—Toxin-positive CDI is more severe, but there were no differences in adjusted CDI-related complication and mortality rates between toxin-positive and NAAT-positive-only CDI that were detected by an algorithm that utilized an initial glutamate dehydrogenase screening test.

Keywords

Clostridioides difficile infection; CDI; diagnostic testing; outcomes

Clostridioides difficile (formerly *Clostridium difficile)* infection (CDI), a toxin-mediated disease, has increased in incidence over the past 2 decades to become the most common US healthcare-associated infection [1], resulting in an estimated 453 000 infections in 2011 [2]. Between 2010 and 2014, CDI-related hospitalizations increased by 15.7% [3]. Increased CDI incidence and severity may be largely explained by the epidemic strain, ribotype 027, that emerged in the early 2000s [4–6]. Another virulent strain, ribotype 078, has been identified mostly in Europe and is associated with serious infections, similar to ribotype 027 [7, 8].

Early in the ribotype 027 epidemic, cases of severe CDI missed by toxin assays and advances in assay development led to the introduction of nucleic acid amplification tests (NAATs), which are highly sensitive for *C. difficile* toxin gene detection [9–12]. Although recent evidence suggests that neither the detectable presence of toxin nor the level of toxin can distinguish *C. difficile* infection from colonization [13, 14], NAAT may be more likely than toxin assays to detect colonization than active infection, since it detects only the presence of the toxin gene rather than the toxin. Some studies have found that toxin-positive CDIs are more severe and have worse outcomes than NAAT-positive-only CDI [15–20], suggesting that reliance on NAAT diagnostic testing might lead to the overdiagnosis of CDI, which could result in unnecessary antibiotic treatment, with disruptive effects on gastrointestinal microbial communities, thereby increasing CDI risk [21]. However, most of the studies comparing toxin and NAAT testing were single-center studies that did not adjust for underlying comorbidities or other potential confounders. One multisite study only detected a difference in mortality outcome in univariate analysis and may have been underpowered to detect a difference in multivariable analysis [20]. Therefore, we sought to conduct a large multisite analysis to compare the clinical course and outcomes of toxin-positive and NAAT-positive-only patients located across diverse geographical areas.

METHODS

CDI Surveillance and Case Definition

Active population-based CDI surveillance is conducted by the Centers for Disease Control and Prevention's (CDC) Emerging Infections Program (EIP) in 10 US sites [22]. Seven of the 10 EIP sites (California, Colorado, Georgia, Maryland, New Mexico, New York, and Tennessee) participated in this analysis. This project underwent ethical review by the CDC and participating EIP sites and their institutional review boards as applicable and was deemed nonresearch by all parties.

Laboratories serving the surveillance catchment areas reported all positive *C. difficile* tests performed during routine clinical care. An incident CDI case was defined as a positive *C. difficile* stool specimen from a person ≥1 year old who had no positive tests in the prior 8 weeks. For this analysis, we included only cases detected from 23 laboratories that utilized the following diagnostic testing algorithm (Figure 1): Specimens were first tested using an enzyme immunoassay (EIA) to simultaneously assess for glutamate dehydrogenase (GDH) and toxin (Techlab C. Diff Quik Chek Complete); specimens positive for both GDH and toxin were considered positive for *C. difficile*, and specimens negative for both were considered negative for *C. difficile* and not tested further. Specimens discordant for GDH and toxin were further tested by NAAT and were considered positive for *C. difficile* if NAAT was positive, and negative for *C. difficile* if NAAT was negative. Based on the testing algorithm results, cases were classified as either toxin positive or NAAT positive only.

Data Collection

An initial records review was performed to collect demographic data and the date and location of stool collection on all CDI cases in 5 EIP sites and on a random sample of cases in 2 EIP sites (Colorado and Georgia) with the largest surveillance populations. Cases were considered community onset if the *C. difficile*-positive stool was collected as an outpatient or within 3 days of hospital admission. All other cases were considered healthcare facility onset.

A subsequent comprehensive chart review was performed on all community-onset cases and a random 10% sample of healthcare facility-onset cases to collect the following information: underlying comorbidities; relevant healthcare and medication exposures; hospitalization at the time of or within 7 days of CDI diagnosis; documentation of diarrhea (including "clinically significant diarrhea," defined as ≥3 unformed stools for ≥1 day); CDI-related complications (defined as toxic megacolon, ileus, colectomy, or admission to the intensive care unit [ICU] on the day of or following CDI diagnosis); CDI treatment; and occurrence of a first recurrent episode (defined as the first positive stool test between 2 and 8 weeks following the initial positive test). The occurrence of death up to 90 days following CDI diagnosis was obtained from the state death registries.

Community-onset cases were further classified as community associated if there was no documentation in the medical record of an overnight stay in a healthcare facility in the preceding 12 weeks. All other community-onset cases and health-care facility-onset cases

were classified as healthcare-associated CDI (includes hospital-onset and long-term care facility-onset cases).

Isolate Collection

Stool specimens from a subset of cases were submitted to the Edward Hines Jr Veterans Affairs Hospital for *C. difficile* culture. Recovered isolates underwent strain typing at CDC using capillary-based polymerase chain reaction ribotyping; results were analyzed against a library of standard profiles using BioNumerics. The proportion of strains that were ribotype 027 and 078 was compared between toxin-positive and NAAT-positive-only patients.

Statistical Analysis

We included all cases with a comprehensive medical record review. Descriptive statistics and unadjusted odds ratios were used to summarize the variables of interest. The χ^2 test and Fisher exact test (where applicable) were used to compare crude results between toxin-positive and NAAT-positive-only cases. Kaplan-Meier curves were used to show the time to death up to 90 days following CDI diagnosis for both groups.

Separate multivariable logistic regression models adjusting for potential confounders—specifically age, sex, race, Charlson comorbidity index, epidemiologic classification, and receipt of oral vancomycin within 3 days of CDI diagnosis—were used to calculate adjusted odds ratios (aORs) to compare toxin-positive and NAAT-positive-only cases for each of the following outcomes: CDI-related complications (defined as above), CDI recurrence (defined as above), and death within 30 days. For the outcome of recurrence, we also adjusted for history of CDI in the prior 6 months. Interaction between epidemiologic classification (community associated vs healthcare associated) and test type was assessed in each multivariable model. Missing race (11.4% of cases) was imputed based on the distribution of known race by age, sex, epidemiologic classification, and EIP site.

Among cases with a recurrent episode, the proportion of each test type for first recurrent episode was compared between toxin-positive and NAAT-positive-only cases.

SAS statistical software version 9.4 (SAS Institute, Cary, North Carolina) and $\alpha = .05$ were used for all analyses.

RESULTS

Of 4878 CDI cases with a comprehensive medical record review, 61.0% were female, and 42.5% were aged ≥65 years. The median number of cases per EIP site was 478 (range, 67–1544), with the largest percentages of cases reported from New York (31.7%), New Mexico (28.4%), and California (16.4%) (Table 1). Overall, 2160 (44.3%) were toxin positive, and 2718 (55.7%) were NAAT positive only. Toxin-positive cases were more likely than NAAT-positive-only cases to be white (73.3% vs 68.4%; $P < .0001$), ≥65 years old (48.2% vs 38.0%; $P < .0001$), and classified as healthcare associated (44.1% vs 35.4%; $P < .0001$).

A larger percentage of toxin-positive than NAAT-positive-only cases had prior hospitalization (39.7% vs 31.8%; $P < .0001$), long-term acute-care hospitalization (0.7% vs

0.2%; $P = .02$), long-term care facility stay (8.7% vs 5.2%; $P < .0001$), and surgery (16.5% vs 13.0%; $P = .0005$) in the 12 weeks preceding their CDI diagnosis (Table 2). Toxin-positive cases were also more likely than NAAT-positive-only cases to have had antibiotic use in the preceding 12 weeks (80.3% vs 65.4%; $P < .0001$).

Among cases with data available, 1853 of 2099 (88.3%) toxin-positive vs 2269 of 2636 (86.1%) NAAT-positive-only cases had documentation of diarrhea ($P = .02$); a greater subset of toxin-positive than NAAT-positive-only cases had ≥3 unformed stools for ≥1 day (813/1853 [43.9%] vs 831/2269 [36.6%]; $P < .0001$). Toxin-positive cases were also more likely than NAAT-positive-only cases to have endoscopic or histologic evidence of pseudomembranous colitis (9/86 [10.5%] vs 2/130 [1.5%]; $P = .008$), white blood cell (WBC) count ≥15 000 cells/μL (483/1539 [31.4%] vs 423/1978 [21.4%]; $P < .0001$), and albumin <2.5 g/dL (333/1207 [27.6%] vs 358/1511 [23.7%]; $P = .02$) (Table 2). There was no difference in frequency of hospitalization at the time of or within 7 days of CDI diagnosis between toxin-positive and NAAT-positive-only community-onset cases (47.7% vs 50.4%; $P = .07$; Table 2).

Among cases with data available, CDI treatment was administered to 1985 of 2055 (96.6%) toxin-positive vs 2442 of 2563 (95.3%) NAAT-positive-only cases ($P = .03$). Oral vancomycin was administered within 3 days of CDI diagnosis in 33.6% of toxin-positive cases vs 30.8% of NAAT-positive-only cases ($P = .05$). Among those with WBC count ≥15 000 cellμL, receipt of oral vancomycin was more common in toxin-positive than NAAT-positive-only cases (56.7% vs 45.4%; $P = .0008$).

Recurrent infection was more frequent among toxin-positive than NAAT-positive-only cases (20.6% vs 11.4%; $P < .0001$) (Table 2). However, there were no differences in unadjusted CDI-related complication and 30-day mortality rates. Unadjusted Kaplan-Meier curves of time to death were similar between the 2 groups (Figure 2). In multivariable analysis, only the outcome of CDI recurrence was significantly associated with toxin positivity (aOR, 1.89; 95% confidence interval, 1.61–2.23; Table 3). We performed a sensitivity analysis on the subset of cases with clinically significant diarrhea (≥3 unformed stools for ≥1 day) and also found that only CDI recurrence remained significantly associated with toxin positivity. We also stratified by onset of disease (ie, analyzed healthcare facility-onset cases separately from community-onset cases) and found the same results.

We sought to understand whether a toxin-positive vs NAAT-positive-only recurrence was associated with a toxin-positive incident case. Of 756 incident cases with recurrent disease, 679 (89.8%; 391 toxin positive, 288 NAAT positive only) had their recurrent episode diagnosed at a laboratory that utilized a toxin EIA, and 77 (10.2%; 54 toxin positive, 23 NAAT positive only) either had no information available on the diagnostic test used for their first recurrent episode or their recurrent episode was diagnosed at a NAAT-only laboratory. Among these 679 incident cases for which a toxin-positive vs NAAT-positive-only recurrence could be determined, 279 of 391 (71.4%) toxin-positive incident cases vs 95 of 288 (33.0%) NAAT-only incident cases had a toxin-positive recurrence ($P < .0001$).

Isolates were available for 282 (13.1%) toxin-positive cases and 323 (11.9%) NAAT-positive-only cases. Of the total 605 isolates, 59 were ribotype 027, and 14 were ribotype 078. A significantly higher proportion of toxin-positive cases (20.6% [n = 58]) than NAAT-positive-only cases (4.6% [n = 15]) were associated with ribotype 027 or 078 ($P < .0001$). Toxin EIA detected 79.5% of strains that were either ribotype 027 or 078 vs 42.1% of all other strain types ($P < .0001$).

DISCUSSION

To our knowledge, this is the largest multisite study to date to compare relevant exposures, clinical and molecular characteristics, and outcomes between toxin-positive and NAAT-positive-only CDI. We found that toxin positivity was significantly associated with prior antibiotic exposure and inpatient and long-term care facility stays. The clinical presentation of toxin-positive patients was more severe by some markers, although toxin positivity was not associated with CDI-related complications. Notably, toxin-positive infections were associated with more virulent *C. difficile* strains and were more likely to recur as toxin-positive again. However, there was no difference in 30-day all-cause mortality between toxin-positive and NAAT-positive-only patients, even after adjusting for several potential confounders.

Several of our findings are consistent with prior studies [15–20] and support the growing evidence that patients who are NAAT positive only might have milder infections and may be more likely to be colonized than toxin-positive patients. We found that NAAT-positive-only patients were significantly less likely to have traditional CDI risk factors, such as prior antibiotic use and recent inpatient healthcare exposures. NAAT-positive-only patients were also less likely to have clinically significant diarrhea and severe disease compared to toxin-positive patients, as demonstrated by fewer cases with markedly elevated WBC count and fewer diagnoses of pseudomembranous colitis. In addition, we found NAAT-positive-only patients were less likely to have recurrent infection. Conversely, toxin-positive patients were more likely to recur with toxin-positive disease again, suggestive of a relapse caused by the same, more virulent strain, although a new infection by another strain is not excluded.

Despite the aforementioned findings, there were no differences in CDI-related complication rates between the 2 groups.

This could be partly due to the overall rarity of toxic megacolon and colectomy, especially in patients treated early in the course of their infections. Comparably low rates of CDI-related complications have been previously described, with no differences observed between toxin-positive and NAAT-positive-only patients [16, 19, 23, 24], despite the higher WBC count observed among toxin-positive patients [16, 19]. In contrast, one study found a significant difference in crude rates of CDI-related complications, where 7.6% of toxin-positive patients had a colectomy, toxic megacolon, or an ICU admission related to CDI, compared to none of the NAAT-positive-only patients [18].

Importantly, we did not find a difference in adjusted 30-day all-cause mortality between toxin-positive and NAAT-positive-only patients. There were also no differences in adjusted

60- and 90-day mortality (data not shown) or in the 90-day survival curves. Almost all of the previous studies that compared mortality rates between these 2 groups did not adjust for potential confounders and have reported conflicting results, with some observing a higher 14- or 30-day all-cause or CDI-related mortality in toxin-positive patients [15, 17, 18], a higher 1-year mortality but not 30-day mortality in toxin-positive patients [16], or no differences in mortality rates [23–27]. Of the few studies that adjusted for potential confounders, one was a single-center study that found a NAAT-positive-only result was independently associated with reduced 30- and 90-day mortality [19]. Another multisite study found a higher 30-day mortality rate among 435 toxin-positive vs 207 cytotoxigenic culture-positive-only patients (comparable to NAAT-positive only) in univariate analysis, but not after adjusting for age, disease severity, and study site [20]; the lack of a significant finding in multivariable analysis has been partly attributed to the study's small sample size [28]. We included 7 times more patients than the other multisite study, found several factors among toxin-positive patients that have previously been associated with poor outcomes (greater frequency of elevated WBC count and ribotype 027 strain) [29, 30], and adjusted for additional important confounders, including underlying comorbidities. The fact that we did not find a significant difference is surprising but might reflect some effect on mortality in NAAT-positive-only patients from receipt of unnecessary CDI treatment [31, 32], given that, in contrast to other studies [18, 20], most patients in our study did receive treatment. The lack of a difference in mortality and complication rates might also be due to a relatively lower prevalence of ribotype 027 during this study period compared to prior periods (9.8% during 2014–2015 vs 28.4% during 2009–2011 [30]). In addition, there might be better management of toxin-positive severe disease (ie, WBC ≥15 000 cells/μL), where more toxin-positive patients with elevated WBC count received oral vancomycin therapy compared to NAAT-positive-only patients.

Although consistent with other studies [18, 25, 26], our finding of more toxin-positive than NAAT-positive-only CDI being associated with ribotypes 027 and 078 has not been widely recognized. We found that toxin EIA was more likely to detect these ribotypes than other strains, likely due to the increased toxin production associated with these virulent strains. A previous study found that the sensitivity of toxin EIA can vary greatly by strain type and that its sensitivity is highest for ribotype 027 (78.4%), whereas strain type has less impact on the sensitivity of NAAT [33], likely due to the fact that NAAT detects the toxin gene rather than toxin production. This finding may have important implications for facilities reporting CDI rates and national burden estimates, where having some knowledge about the prevalence of different strain types could improve our ability to adjust CDI data for test type (toxin EIA vs NAAT).

Our evaluation had several limitations. We could not control for the effect of CDI treatment on outcomes. Since almost all patients were treated, we cannot draw any conclusions on the need for CDI treatment in NAAT-positive-only patients. However, we were able to control for the type of treatment received (ie, vancomycin vs metronidazole), although the inclusion of treatment received did not affect the results from the multivariable model (data not shown). We did not have information on the cause of death and therefore could not determine the proportion of deaths that were attributable to CDI. Among patients with CDI recurrence, 54 toxin-positive and 23 NAAT-positive-only patients could not be assessed for

whether their recurrent episode was toxin positive or NAAT positive only. However, even if we assumed these patients had opposite test results during recurrence, we would still find that toxin-positive CDI is more likely to recur as toxin positive again. In addition, missing data due to incomplete documentation in medical charts might have limited our ability to detect an association for some variables. We also did not account for facility testing practices (eg, if alternative causes of diarrhea, such as laxative use, were excluded before CDI testing) that could have impacted the number of potentially colonized patients included in our analysis. Last, few isolates were available from only 12% of all patients, but this was sufficient to detect an association between toxin positivity and more virulent strains.

In conclusion, our results provide new data on the differences in patients who are diagnosed with one of the guideline-recommended toxin-containing algorithms that specifies NAAT testing only to resolve discordant GDH and toxin EIA results [28]. Although toxin EIA appears to capture more severe disease that is more likely to recur, at least as presently diagnosed, there were no differences in adjusted rates of CDI-related complications and mortality between toxin-positive and NAAT-positive-only CDI. In addition, the low rate of documented clinically significant diarrhea in this analysis suggests that inappropriate testing may be common, highlighting the need for better diagnostic stewardship practices. CDI treatment is not without harm and can result in microbiome disruption; regardless of which test is used, the decision to test and treat should take into consideration the patient's clinical presentation and risk factors. Further advances in CDI diagnostic assays are needed to help determine which patients are likely to experience net benefit from CDI treatment.

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References

1. Magill SS, Edwards JR, Bamberg W et al. Multistate point-prevalence survey of health care-associated infections. N Engl J Med 2014; 370:1198–1208. [PubMed: 24670166]
2. 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. [PubMed: 25714160]
3. Agency for Healthcare Research and Quality. Healthcare cost and utilization project. *Clostridium difficile* hospitalizations 2010–2014. Available at: hcup-us.ahrq.gov/reports/HCUPCDiffHosp2010-2014Report102616.pdf Accessed 12 June 2018.
4. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N Engl J Med 2005; 353:2442–9. [PubMed: 16322602]
5. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N Engl J Med 2005; 353:2433–41. [PubMed: 16322603]
6. Warny M, Pepin J, Fang A, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet 2005; 366:1079–84. [PubMed: 16182895]

7. Keel K, Brazier JS, Post KW, Weese S, Songer JG. Prevalence of PCR ribotypes among *Clostridium difficile* isolates from pigs, calves, and other species. J Clin Microbiol 2007; 45:1963–4. [PubMed: 17428945]
8. Goorhuis A, Bakker D, Corver J, et al. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. Clin Infect Dis 2008; 47:1162–70. [PubMed: 18808358]
9. Dallal RM, Harbrecht BG, Boujoukas AJ, et al. Fulminant *Clostridium difficile:* an underappreciated and increasing cause of death and complications. Ann Surg 2002; 235:363–72. [PubMed: 11882758]
10. Pépin J, Valiquette L, Alary ME, et al. *Clostridium difficile-associated* diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ 2004; 171:466–72.
11. Johal SS, Hammond J, Solomon K, James PD, Mahida YR. *Clostridium difficile* associated diarrhoea in hospitalised patients: onset in the community and hospital and role of flexible sigmoidoscopy. Gut 2004; 53:673–7. [PubMed: 15082585]
12. Burnham CA, Carroll KC. Diagnosis of *Clostridium difficile* infection: an ongoing conundrum for clinicians and for clinical laboratories. Clin Microbiol Rev 2013; 26:604–30. [PubMed: 23824374]
13. Pollock NR, Banz A, Chen X et al. Comparison of *Clostridioides difficile* stool toxin concentrations in adults with symptomatic infection and asymptomatic carriage using an ultrasensitive quantitative immunoassay. Clin Infect Dis 2019; 68:78–86. [PubMed: 29788296]
14. Ng Wong YK, Gonzalez-Orta M, Saldana C, Cadnum JL, Jencson AL, Donskey CJ. Frequency of positive enzyme immunoassay for toxin in stool of asymptomatic carriers of *Clostridium difficile* [manuscript published online ahead of print 22 August 2018]. Clin Infect Dis 2018. doi: 10.1093/cid/ciy701.
15. Baker I, Leeming JP, Reynolds R, Ibrahim I, Darley E. Clinical relevance of a positive molecular test in the diagnosis of *Clostridium difficile* infection. J Hosp Infect 2013; 84:311–5. [PubMed: 23831282]
16. 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. [PubMed: 24813402]
17. 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. [PubMed: 27505006]
18. 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. [PubMed: 26348734]
19. Avni T, Babich T, Ben-Zvi H, et al. Molecular-based diagnosis of *Clostridium difficile* infection is associated with reduced mortality. Eur J Clin Microbiol Infect Dis 2018; 37:1137–42. [PubMed: 29627950]
20. 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. [PubMed: 24007915]
21. Isaac S, Scher JU, Djukovic A, et al. Short- and long-term effects of oral vancomycin on the human intestinal microbiota. J Antimicrob Chemother 2017; 72:128–36. [PubMed: 27707993]
22. Centers for Disease Control and Prevention. Healthcare-associated infections–community interface. *Clostridium difficile* infection tracking. Available at: https://www.cdc.gov/hai/eip/clostridium-difficile.html Accessed 12 June 2018.
23. Longtin Y, Trottier S, Brochu G, et al. Impact of the type of diagnostic assay on *Clostridium difficile* infection and complication rates in a mandatory reporting program. Clin Infect Dis 2013; 56:67–73. [PubMed: 23011147]
24. Origüen J, Corbella L, Orellana MÁ, et al. Comparison of the clinical course of *Clostridium difficile* infection in glutamate dehydrogenase-positive toxin-negative patients diagnosed by PCR to those with a positive toxin test. Clin Microbiol Infect 2018; 24:414–21. [PubMed: 28811244]
25. 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. [PubMed: 22238444]

26. 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. [PubMed: 21765078]
27. Ziegler M, Landsburg D, Pegues D, et al. Clinical characteristics and outcomes of hematologic malignancy patients with positive *Clostridium difficile* toxin immunoassay versus polymerase chain reaction test results. Infect Control Hosp Epidemiol 2018; 39:863–6. [PubMed: 29690940]
28. 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–e48. [PubMed: 29462280]
29. Pépin J, Valiquette L, Alary ME, et al. *Clostridium difficile*–associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ 2004; 171:466–72.
30. See I, Mu Y, Cohen J, et al. NAP1 strain type predicts outcomes from *Clostridium difficile* infection. Clin Infect Dis 2014; 58:1394–400. [PubMed: 24604900]
31. 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. [PubMed: 29136126]
32. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 2013; 13:790–801. [PubMed: 24096337]
33. Tenover FC, Novak-Weekley S, Woods CW, et al. Impact of strain type on detection of toxigenic *Clostridium difficile*: comparison of molecular diagnostic and enzyme immunoassay approaches. J Clin Microbiol 2010; 48:3719–24. [PubMed: 20702676]

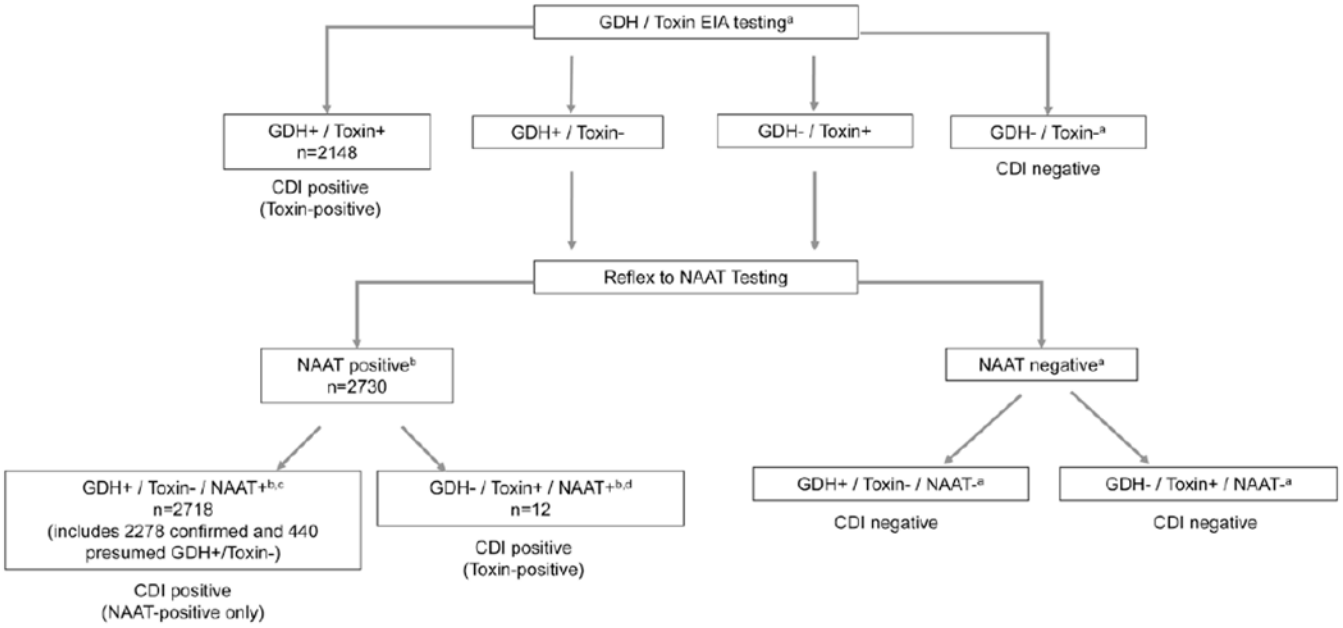

Figure 1.

Clinical laboratory diagnostic testing algorithm for *Clostridioides difficile* infection (CDI). The first step of the testing algorithm consisted of a combined glutamate dehydrogenase (GDH) and toxin enzyme immunoassay (EIA). Specimens positive for both GDH and toxin EIA were considered CDI positive and were reported to Emerging Infections Program (EIP) sites. Specimens negative for both GDH and toxin EIA were considered CDI negative and were not reported to EIP sites. Specimens with discordant GDH and toxin results were reflexed to nucleic acid amplification tests (NAATs). Discordant specimens positive by NAAT were considered CDI positive and were reported to EIP sites. Data regarding the discordant GDH/toxin results (ie, whether cases were GDH positive/toxin negative or GDH negative/toxin positive) were available for 83.9% of these cases (see footnote b). Discordant specimens negative by NAAT were considered CDI negative and were not reported to EIP sites. [a]Since only CDI-positive results were reported to EIP sites, the total number of patients initially tested by GDH and toxin EIA and the total number of patients who tested negative at each step of the algorithm were not available. [b]A total of 2730 discordant GDH/toxin cases had a positive NAAT result; 2290 (83.9%) had data available regarding the results of the GDH/toxin EIA testing, and 440 (16.1%) did not. Of the 2290 cases with data available, 2278 (99.5%) were GDH positive/toxin negative and 12 (0.5%) were GDH negative/toxin positive. [c]2718 GDH-positive/toxin-negative/NAAT-positive cases were classified as NAAT positive only for the analysis. Of these, 2278 were confirmed as GDH positive/toxin negative and 440 were assumed to be GDH positive/toxin negative. We made this assumption based on the increased sensitivity of GDH over toxin EIA and the fact that 99.5% of discordant cases with known GDH/toxin testing results were GDH positive/toxin negative. [d]Twelve cases were GDH negative/toxin positive/NAAT positive; although extremely rare, the negative GDH in these cases might represent a false-negative result. These cases were classified as toxin-positive CDI cases for the analysis.

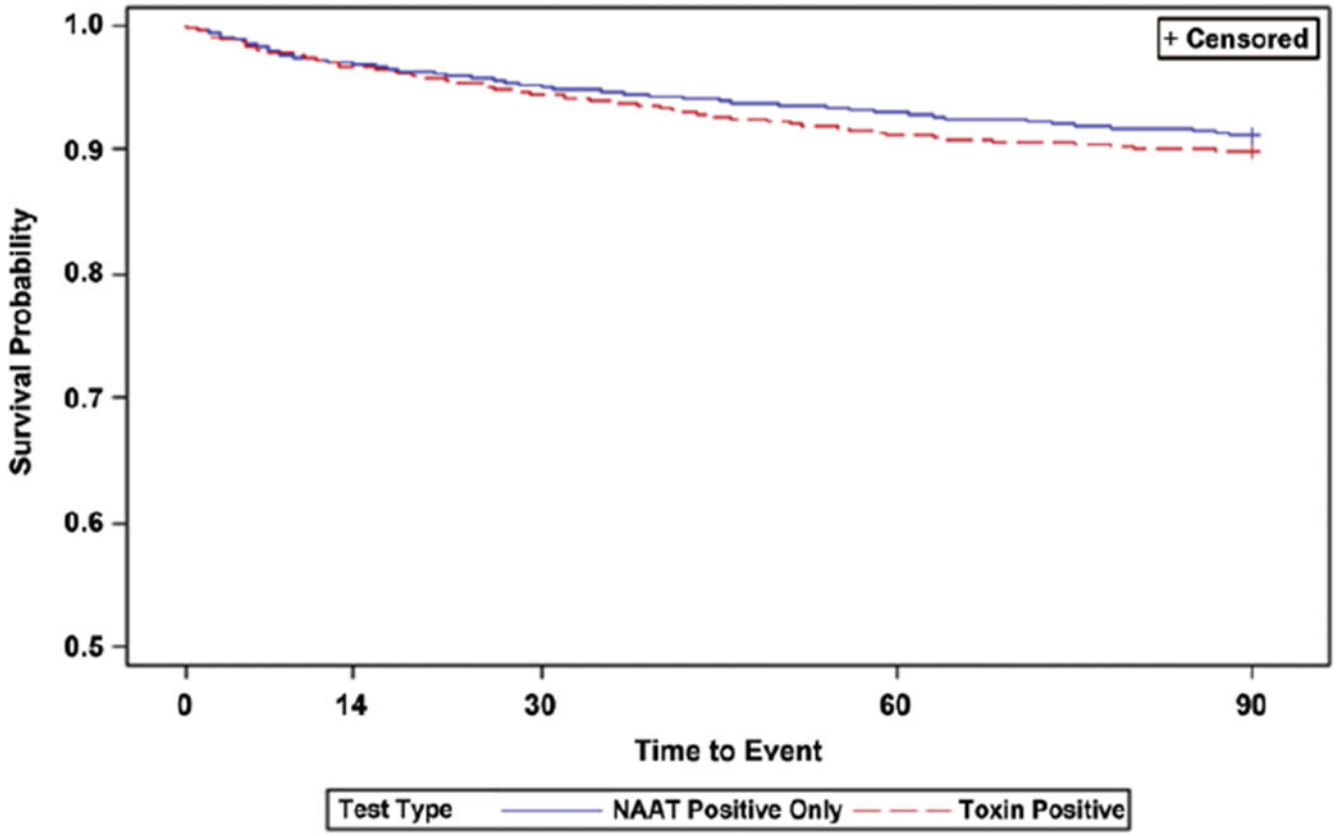

Figure 2.

Unadjusted Kaplan—Meier curves of time to death for toxin-positive and nucleic acid amplification test-positive-only *Clostridioides difficile* infection (CDI) cases. Time to death was followed for up to 90 days following CDI diagnosis for all cases. Abbreviation: NAAT, nucleic acid amplification test.

Table 1.

Comparison of Demographics, Epidemiologic Classification, and Underlying Conditions Between Toxin-positive and Nucleic Acid Amplification Test-positive-only *Clostridioides difficile* infection Cases

Variable	Overall (N = 4878)	Toxin Positive (n = 2160)	NAAT Positive Only (n = 2718)	*P*Value
State				< .0001
California	798 (16.4)	335 (15.5)	463 (170)	
Colorado	323 (6.6)	129 (6.0)	194 (71)	
Georgia	283 (5.8)	102 (4.7)	181 (6.7)	
Maryland	479 (9.8)	222 (10.3)	257 (9.5)	
New Mexico	1384 (28.4)	688 (31.9)	696 (25.6)	
New York	1544 (31.7)	633 (29.3)	911 (33.5)	
Tennessee	67 (1.4)	51 (2.4)	16 (0.6)	
Sex, female	2976 (61.0)	1334 (61.8)	1642 (60.4)	.34
Race				< .0001
White	3442 (70.6)	1584 (73.3)	1858 (68.4)	
Black	585 (12.0)	199 (9.2)	386 (14.2)	
Other	293 (6.0)	108 (5.0)	185 (6.8)	
Unknown	558 (11.4)	269 (12.5)	289 (10.6)	
Age, y				< .0001
1–17	241 (4.9)	86 (4.0)	155 (5.7)	
18–44	1004 (20.6)	359 (16.6)	645 (23.7)	
45–64	1561 (32.0)	675 (31.3)	886 (32.6)	
≥65	2072 (42.5)	1040 (48.2)	1032 (38.0)	
Epidemiologic classification				< .0001
Community associated	2964 (60.8)	1208 (55.9)	1756 (64.6)	
Healthcare associated	1914 (39.2)	952 (44.1)	962 (35.4)	
Charlson comorbidity index				.49
0	1745 (35.8)	773 (35.8)	972 (35.8)	
1	924 (18.9)	410 (19.0)	514 (18.9)	
2	748 (15.3)	318 (14.7)	430 (15.8)	

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Variable	Overall (N = 4878)	Toxin Positive (n = 2160)	NAAT Positive Only (n = 2718)	*P*Value
≥3	1422 (29.2)	637 (29.5)	785 (28.9)	
Unknown	39 (0.8)	22 (1.0)	17 (0.6)	

Data are presented as no. (%) unless otherwise indicated.

Abbreviation: NAAT, nucleic acid amplification test.

Table 2.

Comparison of Risk Factors, Complications, Treatment, and Outcomes Between Toxin-positive and Nucleic Acid Amplification Test-positive-only *Clostridioides difficile* infection Cases

Variable	Overall (N = 4878)	Toxin Positive (n = 2160)	NAAT Positive Only (n = 2718)	Odds Ratio (95% CI)	*P*Value
CDI in previous 6 mo	430 (8.8)	177 (8.2)	253 (9.3)	0.87 (.71–1.06)	.17
Prior healthcare exposures[a]					
Hospitalization	1722 (35.3)	857 (39.7)	865 (31.8)	1.41 (1.25–1.59)	< .0001
Long-term acute care hospitalization	20 (0.4)	14 (0.7)	6 (0.2)	2.95 (1.13–769)	.02
Long-term care facility stay	328 (6.7)	188 (8.7)	140 (5.2)	1.76 (1.40–2.20)	< .0001
Emergency room visit	1243/4855 (25.6)	548/2146 (25.5)	695/2709 (25.7)	0.99 (.87–1.13)	.92
Observational unit stay	103/4854 (2.1)	44/2149 (2.1)	59/2705 (2.2)	0.94 (.63–1.39)	.75
Chronic hemodialysis	229/4854 (4.7)	88/2149 (4.1)	141/2705 (5.2)	0.78 (.59–1.02)	.07
Surgery	704/4846 (14.5)	354/2143 (16.5)	350/2703 (13.0)	1.33 (1.13–1.56)	.0005
Prior medication exposures[a]					
Antibiotics	3441/4779 (72.0)	1705/2124 (80.3)	1736/2655 (65.4)	2.15 (1.89–2.46)	< .0001
Proton pump inhibitor	1989/4796 (41.5)	823/2124 (38.8)	1166/2672 (43.6)	0.82 (.73–.92)	.0006
Histamine-2 receptor blockers	724/4782 (15.1)	314/2119 (14.8)	410/2663 (15.4)	0.96 (.82–1.12)	.58
Immunosuppressive drugs	1272 (26.1)	533 (24.7)	739 (272)	0.88 (.77–.998)	.05
Disease severity and complications					
Pseudomembranous colitis	11/216 (5.1)	9/86 (10.5)	2/130 (1.5)	748 (1.58–35.53)	.008[b]
WBC count ≥15 000 cells/μL	906/3517 (25.8)	483/1539 (31.4)	423/1978 (21.4)	1.68 (1.44–1.96)	< .0001
Serum albumin ≤2.5 g/dL	691/2718 (25.4)	333/1207 (276)	358/1511 (23.7)	1.23 (1.03–1.46)	.02
Radiographic findings of toxic megacolon or ileus	96/1758 (5.5)	47/741 (6.3)	49/1017 (4.8)	1.34 (.89–2.02)	.16
Colectomy	16/4866 (0.3)	8/2153 (0.4)	8/2713 (0.3)	1.26 (.47–3.37)	.64
ICU admission	201/4865 (4.1)	87/2151 (4.0)	114/2714 (4.2)	0.96 (.72–1.28)	.79
Hospitalization among community-onset cases[c]	2290/4656 (49.2)	980/2056 (47.7)	1310/2600 (50.4)	0.90 (.80–1.01)	.07
Treatment					
Received oral vancomycin ≤3 d of CDI diagnosis	1480/4618 (32.1)	690/2055 (33.6)	790/2563 (30.8)	1.13 (1.00–1.28)	.05

Variable	Overall (N = 4878)	Toxin Positive (n = 2160)	NAAT Positive Only (n = 2718)	Odds Ratio (95% CI)	*P*Value
Outcomes					
CDI-related complications[d]	218/1751 (12.5)	97/736 (13.2)	121/1015 (11.9)	1.12 (.84–1.49)	.43
CDI recurrence	756 (15.5)	445 (20.6)	311 (11.4)	2.01 (1.72–2.35)	< .0001
30-day mortality	254 (5.2)	120 (5.6)	134 (4.9)	1.13 (.88–1.46)	.33

Data are presented as no. (%) unless otherwise indicated. Any missing response to a variable was excluded from the denominator.

Abbreviations: CDI, *Clostridioides difficile* infection; CI, confidence interval; ICU, intensive care unit; NAAT, nucleic acid amplification test; WBC, white blood cell.

[a]During the 12 weeks prior to CDI diagnosis.

[b]Fisher exact test.

[c]Excludes 5 cases with missing information regarding hospitalization status.

[d]CDI-related complications defined as toxic megacolon, ileus, colectomy, or ICU admission.

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Table 3.

Multivariable Models Assessing *Clostridioides difficile* infection (CDI)-related Complications, Recurrence, and 30-Day Mortality Among Toxin-positive and Nucleic Acid Amplification Test-positive-only CDI Cases

Outcome	Sample Size, No.	Adjusted Odds Ratio (95% CI) Toxin-Positive vs NAAT-positive Only
CDI-related complications[a]	1749	0.91 (.67–1.23)
CDI recurrence	4599	1.89 (1.61–2.23)
30-day mortality	4599	0.95 (.73–1.24)

Two hundred thirty-nine CDI cases with missing treatment information or Charlson comorbidity index were excluded from the multivariable analyses. Race was imputed for 558 (11%) cases. All models adjusted for age, sex, race, epidemiologic classification, Charlson comorbidity score, and receipt of oral vancomycin within 3 days of CDI diagnosis. The model for CDI recurrence also adjusted for history of CDI in previous 6 months. Interaction between epidemiologic classification and test type was assessed and was nonsignificant in each multivariable model. Abbreviations: CDI, *Clostridioides difficile* infection; CI, confidence interval; NAAT, nucleic acid amplification test.

[a]CDI-related complications defined as toxic megacolon, ileus, colectomy, or intensive care unit admission.