

Does Empirical *Clostridium difficile* Infection (CDI) Therapy Result in False-Negative CDI Diagnostic Test Results?

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Background. Patients with suspected *Clostridium difficile* infection (CDI) often receive empirical therapy prior to collection of stool specimens for diagnostic testing. The likelihood that such empirical therapy might result in false-negative CDI test results is unknown.

Methods. We conducted a prospective study of CDI patients to determine the time to conversion of CDI test results, including polymerase chain reaction (PCR) for toxin B genes, glutamate dehydrogenase, and toxigenic culture, from positive to negative during CDI therapy. We evaluated the frequency of and risk factors for persistence of positive CDI tests. For patients receiving empirical therapy, we assessed the frequency of conversion of positive CDI test results at the time of the test order to negative by the time clinical staff collected stool specimens for testing.

Results. For 51 CDI patients, PCR, glutamate dehydrogenase, and toxigenic culture results converted to negative at similar rates. For PCR, 14%, 35%, and 45% of positive CDI tests converted to negative after 1, 2, and 3 days of treatment, respectively. Increased age and infection with North American pulsed-field gel electrophoresis strains were associated with persistent positive PCR results. For CDI patients diagnosed at the time of the test order, conversion to negative PCR results by the time clinical stool specimens were collected occurred in 4 of 9 (44%) patients who were prescribed empirical CDI therapy versus 0 of 23 (0%) who were not ($P = .004$).

Conclusions. Empirical treatment for suspected CDI cases may result in false-negative PCR results if there are delays in stool specimen collection.

Keywords. *Clostridium difficile*; CDI; diagnosis; empirical treatment.

One of the guiding principles of infectious diseases management is that diagnostic specimens should be collected prior to initiation of antimicrobial therapy whenever feasible. The rationale for this recommendation is that prior antimicrobial therapy may result in false-negative diagnostic test results, particularly cultures [1–5]. Clinicians caring for patients with

suspected *Clostridium difficile* infection (CDI) often prescribe empirical CDI therapy prior to collection of stool specimens for diagnostic testing [6, 7]. The likelihood that such empirical therapy might result in false-negative CDI test results is unknown. False-negative CDI test results could lead to adverse consequences, including progression of disease if CDI therapy is discontinued and transmission if contact precautions are discontinued. This issue has assumed greater importance with the advent of sensitive nucleic acid amplification tests because clinicians now may have increased confidence that a negative CDI test is truly negative [8].

We recently reported that delays in diagnostic testing for CDI were common in our facility (mean time from test order to diagnosis, approximately 2 days), particularly in the long-term-care facility setting [9, 10]. Most of the delay in testing was due to the time required for

Received 2 February 2013; accepted 13 April 2013; electronically published 3 May 2013.

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Clinical Infectious Diseases 2013;57(4):494–500

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DOI: 10.1093/cid/cit286

collection of stool specimens [10]. Others have reported similar delays in CDI diagnostic testing [11]. Here, we tested the hypothesis that delays in collection of stool specimens may increase the likelihood of false-negative CDI test results, including polymerase chain reaction (PCR) for toxin B genes, glutamate dehydrogenase testing, and toxigenic culture, in patients receiving empirical CDI therapy. In addition, we evaluated the frequency of and risk factors for persistence of positive CDI test results.

METHODS

Setting

The Cleveland Veterans Affairs Medical Center includes a 215-bed hospital and 165-bed long-term care facility. During the study, clinical testing for CDI was performed on stool specimens using a commercial polymerase chain reaction (PCR) assay for toxin B genes (Xpert *C. difficile*/Epi Assay, Cepheid, Sunnyvale, California). The hospital's institutional review board approved the study protocol.

Time to Conversion of CDI Test Results From Positive to Negative During CDI Therapy

During a 4-month period, the effect of CDI therapy on CDI test results was determined in consecutive inpatients being treated for CDI on the basis of positive PCR results and diarrhea (ie, ≥ 3 unformed stools in a 24-hour period). Patients who had received empirical CDI therapy prior to the diagnosis were excluded from this analysis. The CDI tests studied included PCR for toxin B genes (Xpert *C. difficile*/Epi assay), enzyme immunoassay for glutamate dehydrogenase (Wampole *C. difficile* Chek-60), and culture for toxigenic *C. difficile*. For each CDI patient, stool specimens or 2 perirectal swabs were collected daily during CDI therapy using BD BBL CultureSwab (Becton Dickinson, Cockeysville, Maryland). The swabs were considered adequate if fecal staining was visible. For the first swab, 1 prong was tested by PCR (Xpert *C. difficile*/Epi assay) according to the manufacturer's protocol, and the other was directly plated onto selective media for culture of toxigenic *C. difficile* as previously described [12]. The second swab was tested by enzyme immunoassay for glutamate dehydrogenase (Wampole *C. difficile* Chek-60); the assay was performed according to the manufacturer's instructions except that, rather than adding 25 μ L of stool suspension, the swabs were vortexed for 10 seconds in the diluent. Stool specimens or swabs were processed within 3 hours of collection. We previously demonstrated that perirectal swabs tested by the Xpert *C. difficile* PCR assay and the glutamate dehydrogenase assay have excellent sensitivity and specificity in comparison to stool specimens in patients with CDI [13]. The Xpert *C. difficile*/Epi assay was used to determine if CDI patients were infected with epidemic North American pulsed-field gel electrophoresis type 1 (ie, NAP1) strains.

Collection of specimens was discontinued when each of the tests converted to negative on 2 consecutive days or after 14 days of CDI therapy or at the time of discharge from the hospital or long-term care facility. Medical records review was performed to obtain information regarding demographics, medical illnesses, severity of CDI, type and duration of CDI treatment, other concurrent antibiotics, and laboratory tests. The ATLAS score was calculated for all patients as described by Miller et al [14], but systemic concomitant antibiotic therapy during CDI treatment ≥ 1 day was substituted for serum creatinine.

Frequency of False-Negative CDI Tests in Patients Receiving Empirical CDI Therapy

To determine if empirical CDI therapy results in false-negative CDI tests in clinical practice, we prospectively enrolled patients diagnosed with CDI based on diarrhea and positive PCR and toxigenic culture results from perirectal swabs collected by research personnel at the time of the order for CDI testing. Patients receiving empirical CDI therapy were enrolled during the 4-month prospective study, and additional patients were included in a prior publication [15]. One prong of the swab was tested by PCR and the other by toxigenic *C. difficile* culture as previously described. Swabs were processed within 3 hours of collection.

Stool specimens for CDI testing were collected by the clinical nursing staff. For patients receiving empirical CDI therapy, the time from the order for empirical therapy to collection of stool specimens by nursing was calculated. Medical records review was performed to obtain information regarding demographics, medical illnesses, severity of CDI, type and duration of CDI treatment, and other concurrent antibiotics and laboratory tests.

Microbiology

Stool specimens or swabs were transferred to an anaerobic chamber (Coy Laboratories, Grass Lake, Michigan) and plated onto prereduced *C. difficile* Brucella agar and processed as previously described [12]. All isolates were tested for in vitro toxin production using *C. difficile* Tox A/B II (Wampole Laboratories); isolates that did not produce toxin were excluded from the analysis.

Data Analysis

For the assessment of the effect of CDI therapy on CDI test results, we examined factors associated with delayed conversion of PCR and toxigenic culture results from positive to negative. Wilcoxon rank-sum test was used to compare median times to conversion across group categories. Kaplan-Meier estimation and curves were used to describe time for positive CDI test results to convert to negative during CDI therapy. The log-rank test was used to compare time to conversion according to type of CDI therapy and by NAP1 versus non-NAP1 strain type. Cox regression was used to explore potential interactions. For

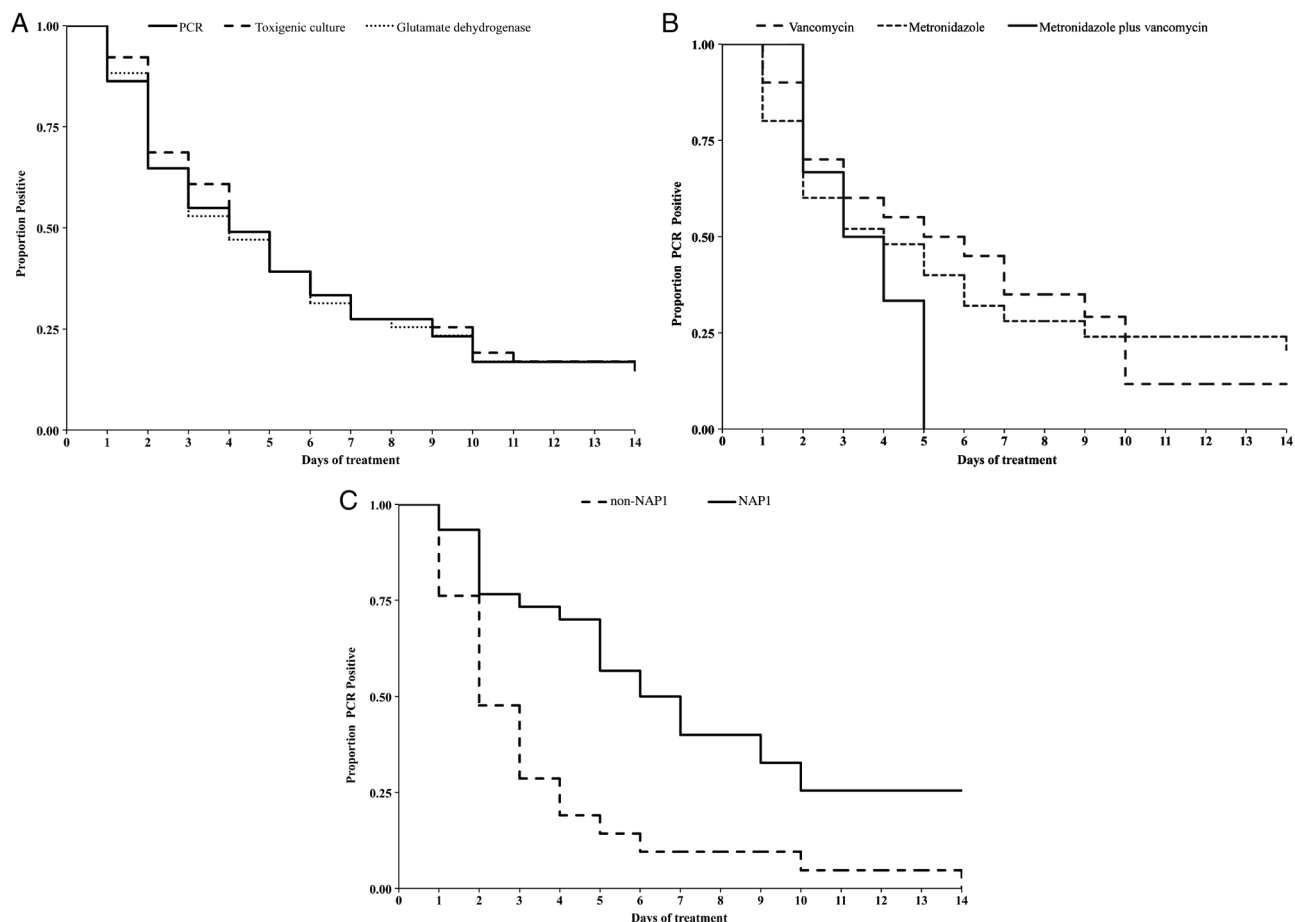


Figure 1. Kaplan-Meier curves showing time to conversion of *Clostridium difficile* infection (CDI) test results from positive to negative during CDI therapy, by test type (toxigenic culture, polymerase chain reaction for toxin B genes, and glutamate dehydrogenase (A), by CDI treatment (metronidazole [n = 25], vancomycin [n = 20], and metronidazole plus vancomycin [n = 6]) (B), and by infection with North American pulsed-field gel electrophoresis type 1 (n = 30) versus non-NAP1 (n = 21) strains (C). Abbreviations: NAP1, North American pulsed-field gel electrophoresis type 1; PCR, polymerase chain reaction.

CDI patients with paired CDI tests at the time of test order and from clinical stool specimens, the proportion converting from positive to negative PCR test results was compared for those who did versus those who did not receive empirical CDI therapy. Because this analysis was exploratory in nature, an a priori calculation of power was not performed. Data were analyzed with the use of SPSS statistical software version 10.0 (SPSS Inc, Chicago, Illinois) and Stata software, version 11 (StataCorp, College Station, Texas).

RESULTS

Time to Conversion of CDI Test Results From Positive to Negative During CDI Therapy

Figure 1A shows the time to conversion of PCR, glutamate dehydrogenase, and toxigenic culture results from positive to

negative during therapy for up to 14 days for 51 CDI patients. The median number of days to convert to negative for each test was 4 days, and the rates of conversion were very similar for each test. After 1, 2, and 3 days of treatment, the cumulative numbers of patients converting from positive to negative PCR test results were 7 (14%), 18 (35%), and 23 (45%) patients, respectively. The rates of conversion to negative were similar in patients treated with metronidazole (n = 25), vancomycin (n = 20), and metronidazole plus vancomycin (n = 6) ($P = .34$; Figure 1B), whereas infection with the NAP1 strain (n = 30 [59%]) was associated with delayed conversion to negative in comparison to non-NAP1 strains ($P < .001$; Figure 1C).

The mean durations of treatment with metronidazole and vancomycin were 14 and 15 days, respectively. Of the 20 patients prescribed oral vancomycin, 4 received vancomycin because they had severe CDI; 11 were on warfarin, which may

have a drug interaction with metronidazole; and 5 had recurrent CDI. Of the 6 patients treated with metronidazole plus vancomycin, 2 had severe, complicated CDI, 1 had an exacerbation of ulcerative colitis in conjunction with CDI, and 3 initially received metronidazole monotherapy with the addition of oral vancomycin due to metronidazole treatment failure.

Table 1 shows a comparison of the characteristics of the 51 CDI patients, according to the time to PCR and toxigenic

culture negativity. In addition to infection with the NAP1 strain, age >69 years was the only factor associated with significant delays in conversion from positive to negative test results. There were more individuals aged >65 in the NAP1 versus non-NAP1 groups, but the difference was not statistically significant (70% vs 57%, respectively; $P = .34$). Moreover, by Cox regression analysis, there was no interaction between increased age and infection with the NAP1 strain ($P = .57$).

Table 1. Characteristics of the 51 *Clostridium difficile* Infection Patients, According to the Time to Polymerase Chain Reaction and Culture Negativity

Characteristic	No. (%)	Median (IQR) Time to PCR Negativity	<i>P</i> Value ^a	Median (IQR) Time to Culture Negativity	<i>P</i> Value ^a
All patients	51 (100)	4 (2–9)	...	4 (2–9)	...
Age, y					
<64	13 (25.5)	2 (2–5)	.01	3 (2–5)	.03
64–68	15 (29.4)	4 (2–7)		4 (2–7)	
≥69	23 (45.1)	6 (2–10)		6 (2–11)	
Strain type					
Non-NAP1	21 (41.2)	2 (1.5–4)	<.001	3 (2–4)	<.001
NAP1	30 (58.8)	6.5 (3–10)		6.5 (4–11)	
Infection severity ^b					
Nonsevere	45 (88.2)	4 (2–8)	.71	4.5 (2–9.5)	.85
Severe	6 (11.8)	4.5 (2–8.5)		4 (2–9)	
Treatment					
Metronidazole	25 (49.0)	4.5 (2–11)	.34	4.5 (2–11.5)	.33
Vancomycin	20 (39.2)	5.5 (2–9.5)		5.5 (2–10)	
Metronidazole plus vancomycin	6 (11.8)	3.5 (2–5)		4 (2–5)	
Braden mobility ^c					
4	10 (19.6)	4.5 (2–7)	.53	4.5 (27)	.57
3	24 (47.1)	3 (1.5–6.5)		3 (2–6.5)	
2	11 (21.6)	7 (5–14)		7 (5–14)	
1	6 (11.8)	4.5 (2–15)		4.5 (2–15)	
Albumin level					
≥2.5 mg/dL	20 (39.2)	2 (2–7)	.35	3.5 (2–7)	.37
<2.5 mg/dL	31 (60.8)	5 (3–10)		5 (3–10)	
ATLAS score ^d					
≤5	41 (80.4)	3 (2–7)	.32	4 (2–7)	.32
>5	10 (19.6)	7 (4–15)		7.5 (4–15)	
Concomitant non-CDI antibiotics					
No	22 (43.1)	5 (2–7)	.84	5 (2–7)	.84
Yes	29 (56.9)	4 (2–9.5)		4 (2–10)	

Data are No. (%) of patients, unless otherwise indicated.

Abbreviations: CDI, *Clostridium difficile* infection; IQR, interquartile range; NAP1, North American pulsed-field gel electrophoresis type 1; PCR, polymerase chain reaction.

^a Wilcoxon rank-sum test was used to compare median times to conversion across group categories.

^b Severe CDI was defined as a case associated with leukocytosis with white blood cell count of ≥15 000 cells/μL or a serum creatinine ≥1.5-fold above the premorbid value [16].

^c Mobility score is a subcategory of the Braden score for prediction of pressure ulcer risk (1 = completely immobile; 2 = very limited; 3 = slightly limited; 4 = no limitation).

^d ATLAS score calculated based on: age: <60 y = 0 points, 60–79 y = 1 point, ≥80 = 2 points; temperature (°C): ≤37.5 = 0 points, 37.6–38.5 = 1 point, ≥38.6 = 2 points; leukocytosis (cells/μL): <16 000 = 0 points, 16 000–25 000 = 1 point, >25 000 = 2 points; albumin (mg/dL): >3.5 = 0 points, 2.6–3.5 = 1 point, ≤2.5 = 2 points; and systemic concomitant antibiotics during CDI treatment (≥1 day), use of systemic antibiotics at time of diagnosis = 2 points [14].

Frequency of False-Negative CDI Tests in Patients Receiving Empirical CDI Therapy

Thirty-two patients were diagnosed with CDI on the basis of perirectal swab specimens collected at the time of the order for CDI testing by research staff and subsequently had CDI testing performed on stool specimens collected by the clinical nursing staff. All perirectal swab specimens that were positive by PCR were also positive by toxigenic culture. Of the 32 CDI patients, 9 (28%) were prescribed empirical CDI therapy and 23 (72%) were not (Figure 2). Four of 9 (44%) empirically treated CDI patients converted to negative PCR and toxigenic culture results compared to 0 of 23 CDI patients who did not receive empirical CDI therapy ($P = .004$). For the 9 patients receiving empirical CDI therapy, the mean time from the start of CDI therapy to collection of a stool specimen by nursing staff was 24 hours (range, 6 hours to 9 days). All 4 of the empirically treated patients whose tests converted to negative had received at least 24 hours of CDI therapy prior to stool collection (range, 1–5 days of CDI therapy prior to stool collection). For the 5 empirically treated patients whose tests did not convert to negative, the mean time from the start of CDI therapy to collection of a stool specimen by nursing staff was 82 hours (range, 6 hours to 9 days).

DISCUSSION

Current guidelines for management of CDI recommend empirical treatment without waiting for test results in patients suspected to have severe CDI [16]. In practice, it is not uncommon

for clinicians to prescribe empirical therapy for patients with suspected CDI and mild to moderate symptoms if it is anticipated that there may be delays in collection and processing of CDI tests [7, 10]. Here, we demonstrated that CDI therapy results in conversion of CDI test results from positive to negative in a significant proportion of patients within 1–3 days. For PCR test results, 14%, 35%, and 45% of positive CDI tests converted to negative after 1, 2, and 3 days of treatment, respectively. In addition, 4 of 9 (44%) CDI patients receiving empirical therapy converted to negative PCR and toxigenic culture results by the time stool specimens were collected for clinical testing from 1 to 5 days after initiation of therapy. These findings have important implications for diagnosis and management of CDI.

False-negative CDI test results associated with empirical therapy could result in several potential adverse consequences. First, patients with CDI may experience progression of their illness or increased risk for recurrence if CDI therapy is discontinued due to a false-negative test. Second, failure to identify CDI patients may result in increased risk for transmission if contact precautions are not instituted or are discontinued prior to resolution of symptoms. Third, the incidence of CDI in an institution may be underestimated if frequent prescription of empirical therapy results in a significant number of false-negative tests. Fourth, false-negative CDI tests may result in unnecessary laboratory testing or procedures to evaluate for alternative explanations for diarrhea. Finally, false-negative tests in patients who respond to CDI therapy may undermine clinicians' confidence in the sensitivity of CDI tests.

Clinicians managing patients with suspected CDI should be aware that empirical therapy may result in false-negative CDI test results. For patients who are prescribed empirical therapy for suspected CDI with mild to moderate symptoms, it might be reasonable to stipulate that empirical therapy should only begin after a stool specimen for testing has been collected. If immediate empirical therapy is indicated for suspected severe CDI, efforts should be made to expedite collection of stool specimens for diagnostic testing. One potential option to expedite testing could be collection of perirectal swabs for carefully selected patients because this method is rapid and provides excellent sensitivity in comparison to stool specimens for diagnosis of CDI [13].

The rapid conversion of CDI test results to negative during therapy is consistent with some previous studies that demonstrated that *C. difficile* concentrations and/or toxin levels may decrease rapidly with effective therapy [17–19]. For example, Louie et al [17] found that oral vancomycin and oral fidaxomicin suppressed *C. difficile* concentrations and cytotoxin B titers to undetectable levels by day 4 of CDI treatment. Similarly, Sethi et al [18] demonstrated that the mean concentration of *C. difficile* in stool decreased from 5.4 log₁₀ colony-forming units (CFU)/g of stool prior to treatment to 3.6 log₁₀ CFU/g of stool

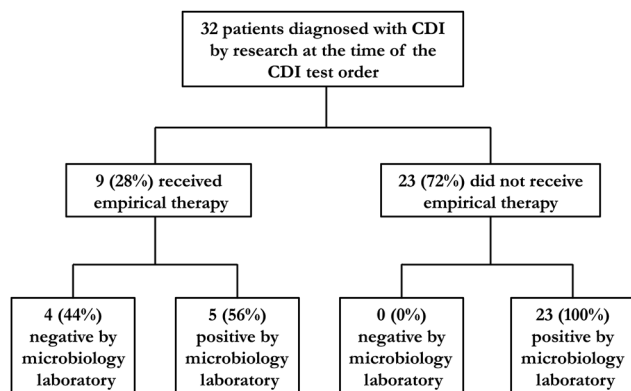


Figure 2. Frequency of false-negative *Clostridium difficile* infection (CDI) polymerase chain reaction (PCR) tests by the clinical microbiology laboratory in 32 patients receiving empirical CDI therapy and diagnosed with CDI at the time of the order for CDI testing based upon perirectal swab specimens collected by research personnel. Clinical CDI testing was performed on stool specimens collected by the clinical nursing staff. All perirectal swab specimens that were positive by PCR were also positive by toxigenic culture. Abbreviation: CDI, *Clostridium difficile* infection.

after 3 days of CDI therapy. We are not aware of previous studies that have evaluated the impact of CDI treatment on results of commercial PCR-based CDI assays. Although PCR-based CDI assays are very sensitive for diagnosis of patients with acute CDI when concentrations of vegetative organisms are high [13, 20], the sensitivity of these assays may not be sufficient for the detection of lower concentrations of *C. difficile* [13, 21].

We found that age >69 years and infection with the NAP1 strain were associated with significant delays in conversion of CDI test results from positive to negative. Patients infected with the NAP1 strain tended to be older than those infected with non-NAP1 strains, but the difference was not statistically significant and there was no interaction between increased age and the NAP1 strain. The clinical significance of these associations is unclear. However, it is notable that increased age and infection with NAP1 strains have been associated with increased risk for recurrence of CDI and decreased likelihood of clinical cure [22–24]. It is plausible that slower or less consistent clearance of *C. difficile* from the intestinal tract might contribute to the increased risk of recurrence and decreased likelihood of cure. Longer courses of CDI therapy such as vancomycin tapers could potentially be beneficial if they provide more time to clear residual *C. difficile* from the intestinal tract. Although 2 prior studies have provided evidence that metronidazole therapy may result in slower and less consistent suppression of *C. difficile* than vancomycin therapy [25, 26], we did not detect a significant difference between these agents in the rate of conversion from positive to negative CDI tests. Finally, because persistent positive CDI test results were common during treatment, our results provide support for the recommendation that repeat testing during the same episode of diarrhea is of limited value and should be discouraged [16].

Our study has some limitations. The study was conducted in a single medical center that cares for mostly male patients. Outpatients were not included in the study. For the assessment of time to conversion of CDI test results to negative, the small number of study subjects may have limited our power to detect an interaction between age and infection with the NAP1 strain. Although patients prescribed empirical CDI therapy were significantly more likely to convert from positive to negative PCR results, the number of CDI patients with paired CDI tests was small. Therefore, there is a need for larger studies to confirm our findings.

Notes

Financial support. This work was supported by the Department of Veterans Affairs (Merit Review grant to C. J. D.).

Potential conflicts of interest. C. J. D. is a consultant for Optimer, GOJO, and 3M and has received research grants from Pfizer, Merck, and Cubist. 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.

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