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Searching for a Potential Algorithm for *Clostridium difficile* Testing at a Tertiary Care Hospital: Does Toxin Enzyme Immunoassay Testing Help?

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ABSTRACT *Clostridium difficile* is a major contributor to morbidity and mortality in the United States. Methods for identifying the organism in stool include molecular platforms, enzyme immunoassays (EIAs) for toxin, and culture. Controversy persists over whether molecular tests are too sensitive at identifying *C. difficile*, and there are questions about how additional laboratory information could inform clinical management and reduce over treatment. The aim of this study was to assess whether clinical factors are related to the toxin status of patients and whether information about toxin status could potentially inform clinical management of patients. A total of 201 PCR-positive *C. difficile* stool samples from adult patients at our institution underwent EIA toxin testing. Clinical and laboratory data were collected, and the percentage of PCR-positive/EIA-positive (PCR^+/EIA^+) patients and PCR^+ and EIA-negative (PCR^+/EIA^-) patients was calculated. Of the 201 samples, 47% were EIA positive and 53% were EIA negative. Although PCR^+/EIA^+ patients were more likely to have had a prior *C. difficile* infection ($P = 0.015$), there was no statistical difference between the additional data collected that correlated with a positive EIA result. We were unable to show that patients with an EIA^+ result had worse clinical parameters than those with EIA^- results and concluded that establishing a testing algorithm that included both PCR and EIA testing would not change the clinical management of patients at our hospital.

KEYWORDS *Clostridioides difficile*, *Clostridium difficile*, *Clostridium difficile* toxin, molecular methods, testing

Clostridium difficile, the causative agent for antimicrobial-associated diarrhea, colitis, and pseudomembranous colitis, is a major contributor to morbidity and mortality in the United States. The CDC reports that it caused half a million infections in the United States in 2011, with attributable costs as high as $4.8 billion for acute care facilities alone (1, 2). Since 2000, there has been a marked increase in *C. difficile* infections and mortality attributed to both newer hypervirulent strains and changes in testing from less sensitive enzyme immunoassays (EIA) to more sensitive nucleic acid amplification test (NAAT) methods (3–7). Specifically, there was a greater than 50% increase in *C. difficile* infection incidence with hospitals that switched from EIA testing to NAAT (5, 8).

It is important, however, that only toxin-producing strains of *C. difficile* cause infections but the presence of toxin-producing *C. difficile* does not equate to diarrheal disease. It has been estimated that up to 7 to 15% of healthy adults are colonized with toxigenic *C. difficile*, with higher rates among residents in long-term-care facilities (9–11). The patient's endogenous microbiota must be disturbed, which is usually accomplished through various antimicrobial agents, in order for *C. difficile* to proliferate (12, 13). In addition, the sequelae of *C. difficile* infections are variable, with a wide

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variation in symptomatology depending on various host/pathogen factors. Infection can vary greatly from asymptomatic carriage to toxic megacolon and even death. Therefore, the presence of toxigenic *C. difficile* strains as detected by molecularly based tests does not necessarily translate to *C. difficile*-associated diarrhea. This point is especially important in hospitalized individuals who may have other etiologies for their diarrheal disease.

Diagnosis of *C. difficile* infection (CDI) is a clinical one, with appropriate signs and symptoms supported by testing for the toxigenic strain of the organism. There are several methods for testing for the toxigenic strains of *C. difficile*, including molecular tests for toxin-producing genes, EIAs for toxin antigen, and culture. Recently, many studies have shown that molecular platforms may be too sensitive, detecting toxigenic *C. difficile* colonization rather than actual disease (8, 14, 15, 16).

New recommendations in the 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) propose three testing strategies when there are no preagreed institutional criteria for patient stool submission: glutamate dehydrogenase (GDH) plus a toxin EIA, GDH plus a toxin EIA arbitrated by an NAAT, or NAAT plus a toxin EIA rather than an NAAT alone (19). The recommendations state that NAAT may be use alone if there are preagreed institutional criteria such as rejecting samples from patients who are on laxatives and testing stool specimens only from patients with new-onset, unexplained diarrhea, defined as $\geq$3 unformed stools in 24 h (19).

We started our study prior to the new recommendations with the intention to identify whether an algorithmic approach to *C. difficile* testing would be more appropriate for our institution than using molecular testing alone. The purpose of this study was to determine what percentage of patients who had toxigenic *C. difficile* as determined by the molecular methodology (PCR-based assay) also had detectable toxin using an immunoassay (ImmunoCard Toxins A & B; Meridian Bioscience, Inc., Cincinnati, OH). *C. difficile* toxin A and B testing was performed on all samples that were positive by PCR (PCR^+) testing. Patient demographics and clinical information were collected on patients to determine whether certain clinical variables were more likely to be associated with both positive PCR and positive EIA (EIA^+) toxin result.

MATERIALS AND METHODS

Study design and population. Both hospitalized patients and outpatients between the ages of 18 and 99 years with diarrheal stool samples positive for *C. difficile* testing by PCR at the University of Vermont Medical Center (UVMMC) between 1 June 2016 and 10 May 2017 were included in the study. Diarrheal stool was defined as a score of $>$4 on the Bristol stool chart. Samples that had a positive *C. difficile* PCR result underwent testing for toxin production by an enzyme immunoassay method. The study protocol (IRB 16-623) was approved by the Administrative Operations Research Protections Office at the University of Vermont.

Laboratory testing. All stool samples were tested with a U.S. Food and Drug Administration (FDA)-approved *C. difficile* molecular test, the BD Max Cdiff assay. Formed stools were rejected as inappropriate for testing (score of 4 or less on the Bristol stool chart). For efficiency, PCR-positive samples were frozen at -20°C to be held for batch enzyme immunoassay testing. According to the test package insert, samples should be stored refrigerated at 2 to 8°C for up to 4 days before testing or stored frozen at -20°C (17). If samples were not frozen immediately after PCR testing, they were refrigerated (2 to 8°C). This was done and noted on 26 samples. Since refrigerated specimens that were frozen after 4 days still tested positive for toxin, we allowed a maximum time without freezing a sample of 7 days. Eleven of our 26 samples were refrigerated between 4 and 7 days. Before toxin testing, samples were allowed to come to room temperature, and the Bristol stool chart was utilized to categorize the stool consistency after samples thawed. Samples then underwent an FDA-approved toxin immunoassay (Meridian ImmunoCard Toxins A & B assay). Testing was performed according to the package insert and manufacturer's instructions (18).

Clinical data collection. At our institution, all patients (both inpatients and outpatients) with positive *C. difficile* PCR tests are included in the population in the infection prevention software system, Theradoc (Premier Incorporated, Charlotte, NC). As part of the infection prevention surveillance, the patient's chart is accessed to view clinical status and hospital encounters. For this study, the following clinical information was collected: white blood cell (WBC) count greater than 11,000/mm^3, prior CDI, temperature of $\geq$38°C or $<$38°C, duration of diarrhea, creatinine level, computed tomography (CT) imaging, laxative use, antibiotic use within the past 2 weeks, class of antibiotic, history of inflammatory bowel disease (IBD), whether or not the patient was immunocompromised, history of enteral feeding, and stool frequency. Prior CDI was defined as having a positive PCR result at any point in the past, with

the majority of patients with recurrent CDI having had a prior CDI within the last year. Immunocompromised status included patients who were diagnosed with HIV/AIDS or cancer or who had a transplant (either solid organ or bone marrow). The Charleston comorbidity index was utilized to correlate clinical conditions with an associated score (20). The conditions included in the ranking included multiple illnesses, most notably the following: myocardial infarct, congestive heart failure (CHF), vascular disease, dementia, diabetes, chronic kidney disease (CKD), solid tumor, hematologic malignancy, liver disease, and HIV/AIDS.

Statistical analysis. The percentages of PCR-positive and toxin-positive patients and of PCR-positive/toxin-negative patients were calculated. Data were analyzed using the JMP statistical package from SAS Institute, Inc., Cary, NC. For categorical variables of symptoms (fever, diarrhea duration, and stool frequency), treatment (recent antibiotic use, recent cephalosporin use, and recent quinolone use), and history (laxative use, tube feeding, prior CDI, history of inflammatory bowel disease, and status as immunocompromised), a chi-square test was performed to compare proportions between the two groups (toxin positive or negative). For continuous variables (WBC count, stool consistency, creatinine level, and age), a *t* test was done to compare means between the two groups (toxin positive or toxin negative). We also performed a binary logistical regression using the variables of immunocompromised status, diarrhea duration, WBC count, stool frequency, prior CDI, prior antibiotic use, and fever as predictors to see whether any of these variables were important in predicting the probability of a toxin-positive EIA.

RESULTS

A total of 201 adult patients that were PCR positive for *C. difficile* (99 outpatient and 102 inpatient) were analyzed over a 12-month period. Women made up 47% of the study population, and the average age was 59 years. The percentages of PCR^+ stools that were EIA^+ and EIA negative (EIA^-) were calculated. We also compared PCR^+/EIA^+ and PCR^+/EIA^- groups to determine if there was a difference in terms of clinical history and symptomatology between them. Factors such as age, stool consistency (average Bristol score, 6.4; standard deviation [SD], 0.9), prior antibiotic administration, creatinine values, and comorbidities were similar among the two groups. There was not enough power to compare differences between inpatient and outpatient samples, so groups were analyzed together.

Of all the *C. difficile* PCR-positive stool samples, 94 (47%) were toxin antigen positive, and 107 (53%) were toxin antigen negative. Although PCR^+/EIA^+ patients were more likely to have had a prior *C. difficile* infection ($P = 0.015$), there was no statistically significant difference between the additional demographic variables, including IBD status or immunocompromised status, and, further, none of the signs/symptoms on which data were collected showed any statistically significant difference with having a positive EIA toxin test.

Forty-three patients with a history of laxative use within the past 24 h were found to be EIA positive. Notably, 45% of patients who did not have documentation of antibiotics in the prior 2 weeks were EIA positive. A summary of the history, symptoms, and treatment results can be seen in Table 1. Of the six patients with CDI complications (colectomy or death), one tested EIA negative, with death as the resulting complication, and the remainder were EIA positive. Of the patients with a stool frequency of less than 3 per day, 33% were found to be EIA positive, and of those receiving tube feedings within 24 h, 38% were found to be EIA positive.

On binary logistic regression, no model including clinical parameters (i.e., fever, duration of diarrhea, and stool frequency) was able to predict whether or not the patient was EIA positive. A cluster analysis did not identify a symptom pattern in PCR^+/EIA^+ patients.

DISCUSSION

Results of our study have been shared with the infection preventionists and infectious disease clinicians at our hospital. Only approximately half (47%) of the PCR-positive samples were EIA positive. As definitive diagnosis of CDI is difficult to ascertain from our data, this could be interpreted to mean that adding EIA to an algorithmic approach after a positive PCR could result in underdiagnosing CDI in approximately half of our patients. Conversely, this result could be interpreted to indicate that using PCR alone overdiagnoses CDI by 50%. Additionally, none of the clinical factors on which we collected data except prior *C. difficile* infection were able to predict EIA positivity. We

TABLE 1 A summary of variables with their corresponding *P* values

Variable class and name	Category[b]	N[c]	% toxin positive	Relative risk	*P* value[d]
History					
Prior CDI[a]	Previous CDI	51	63	1.5	**.015**
	No previous CDI	109	42		
IBD	Diagnosis of IBD	20	35	0.7	.207
	No diagnosis of IBD	132	50		
Immunocompromised	Immunocompromised	44	50	1.0	.760
	Not immunocompromised	110	47		
Symptoms					
Fever	Temp ≥38°C	51	59	1.4	.077
	Temp <38°C	90	43		
Duration of diarrhea	≥2 Days	139	50	1.6	.142
	<2 Days	16	31		
Stool frequency	≥3 Loose stools per day	135	50	1.5	.142
	<3 Loose stools per day	21	33		
Treatment					
Laxative	Laxative use within 24 h of stool collection	23	43	0.9	.632
	No laxative within 24 h of stool collection	133	49		
Recent antibiotic	Antibiotic use in the prior 2 weeks	104	52	1.1	.472
	No antibiotic use in the prior 2 weeks	44	45		
Recent cephalosporin	Cephalosporin use in the prior 2 weeks	35	57	1.3	.215
	No cephalosporin use in the prior 2 weeks	85	45		
Recent quinolone	Quinolone use in the prior 2 weeks	15	60	1.3	.333
	No quinolone use in the prior 2 weeks	105	47		
Enteral nutrition	Tube feeding within 24 h of stool collection	8	38	0.8	.537
	No tube feeding within 24 h of stool collection	146	49		

[a]Prior *C. difficile* infection was defined as having a positive PCR result at any point in the past, with the majority of patients with recurrent CDI having a prior CDI within the last year.
[b]IBD, Inflammatory bowel disease.
[c]*N*, number of patients.
[d]Values in boldface are significant.

did not find a difference for the variables immunocompromised status, having a diagnosis of inflammatory bowel disease, or lack of both diagnoses between EIA-positive and EIA-negative samples. Symptoms such as fever, having a high white blood cell count, duration of diarrhea, and stool frequency did not show any differences between patients with PCR^+/EIA^+ results and those having only PCR-positive results. Having a previously positive *C. difficile* test was the only variable in the patient's history that would indicate a PCR^+/EIA^+ result. Therefore, our institution was unable to establish a testing algorithm based on these data that would have increased the positive predictive value of the molecular testing methodology.

Interestingly, laxative use or recent antibiotic use (and specifically having a recent cephalosporin or quinolone) did not show any differences between a PCR^+/EIA^+ samples and PCR^+/EIA^- samples. Some laboratories do not test samples from patients with a history of laxative use, and therefore they may be missing *C. difficile* infections (21). In addition, having fewer than 3 stools per day and having enteral tube feeding within 24 h did not necessarily mean that the patient did not have an EIA-positive test (33% and 38% EIA positive, respectively). It is important to mention that both the lack of laxative use and greater than three stools per day are now criteria used in the new recommended guidelines proposed by the IDSA and SHEA in which NAAT testing alone is acceptable; however, our results identified a small subset of patients as PCR^+/EIA^+ and having fewer than 3 stools in a 24-h period (19). There was also no difference between patients on laxatives who were EIA positive and those who were EIA negative. Therefore, we concluded that an algorithm using these two pieces of patient history would not be helpful in determining CDI in our institution.

Importantly, one patient with a CDI complication resulting in death was EIA negative in our study. This indicates that if only EIA testing was used by our institution, important

CDI-associated complications would be missed. Our study demonstrated results similar to those of Polage et al. in which one CDI complication (death) was missed (0.6%) if only EIA toxin testing was used (14). Our study method varied by testing *C. difficile* PCR-positive patients for toxin by EIA, whereas Polage and colleagues performed PCR and EIA on all specimens concurrently (14).

Our study is unique in that we were trying to design an algorithm based on specific clinical data that would be useful to determine whether or not *C. difficile* testing would be warranted. Current recommendations by IDSA and SHEA differentiate whether or not to use multistep testing or NAAT alone if there are preagreed criteria for stool samples (19). These include laxative use and having new-onset diarrhea, defined as >3 unformed stools in 24 h (19). Our study showed no difference between PCR^+/EIA^+ and PCR^+/EIA^- patient groups and these two criteria as well as other symptoms/history. We also showed that PCR-positive samples were split between toxin-negative and toxin-positive EIA results. This finding is similar to findings of other studies that showed toxin EIA testing to have decreased sensitivity compared to that of PCR testing (22–24).

Limitations of this study include the retrospective nature of chart reviews which precludes extraction of a full data set for each patient, especially from outpatients. In addition, we did not test any PCR-negative patients with the EIA. It is possible that some PCR-negative samples could be toxin positive, and thus we would have missed these cases. In addition, EIA testing was delayed and did not occur concurrently with PCR. Since some samples were refrigerated for more than 4 days, there is a possibility that the results may have been altered or skewed toward negative immunoassay results since one of the limitations stated in the package insert for the EIA is that samples should be refrigerated for 4 days or less. Our proportion of PCR^+/EIA^- samples, however, was very similar to what has been previously described (53% versus 55.3%) (14).

Our results show that designing an algorithm for *C. difficile* testing using the above symptoms and/or history may lead to missing a CDI and result in unfavorable outcomes for patients. This study also confirmed there were EIA-positive cases in patients who received laxatives and had fewer than three stools in 24 h. In addition, EIA testing for toxin did not capture the one CDI-related death. Until a testing algorithm is designed that increases the positive predictive value of CDIs and/or until more data are collected to establish the best possible testing for *C. difficile*, our laboratory will continue to use PCR as a stand-alone test (25, 26).

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REFERENCES

1. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC. 2015. Burden of Clostridium difficile infection in the United States. N Engl J Med 372:825–834. https://doi.org/10.1056/NEJMoa1408913.
2. CDC. 2017. Healthcare-associated infections: Clostridium difficile infection. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/hai/organisms/cdiff/cdiff_infect.html. Accessed 3/7/2018.
3. Freeman J, Bauer MP, Baines SD, Corver J, Fawley WN, Goorhuis B, Kuijper EJ, Wilcox MH. 2010. The changing epidemiology of Clostridium difficile infections. Clin Microbiol Rev 23:529–549. https://doi.org/10.1128/CMR.00082-09.
4. Kang J, Sickbert-Bennett EE, Brown VM, Weber DJ, Rutala WA. 2014. Changes in the incidence of health care-associated pathogens at a university hospital from 2005 to 2011. Am J Infect Control 42:770–775. https://doi.org/10.1016/j.ajic.2014.03.019.
5. Longtin Y, Trottier S, Brochu G, Paquet-Bolduc B, Garenc C, Loungnarath V, Beaulieu C, Goulet D, Longtin J. 2013. Impact of the type of diagnostic assay on Clostridium difficile infection and complication rates in a mandatory reporting program. Clin Infect Dis 56:67–73. https://doi.org/10.1093/cid/cis840.
6. Zilberberg MD, Shorr AF, Kollef MH. 2008. Increase in adult Clostridium difficile-related hospitalizations and case-fatality rate, United States, 2000–2005. Emerg Infect Dis 14:929–931. https://doi.org/10.3201/eid1406.071447.
7. Lessa FC, Gould CV, McDonald LC. 2012. Current status of Clostridium difficile infection epidemiology. Clin Infect Dis 55(Suppl 2):S65–S70. https://doi.org/10.1093/cid/cis319.
8. Gould CV, Edwards JR, Cohen J, Bamberg WM, Clark LA, Farley MM, Johnston H, Nadle J, Winston L, Gerding DN, McDonald LC, Lessa FC, Clostridium difficile Infection Surveillance Investigators, Centers for Disease Control and Prevention. 2013. Effect of Nucleic acid amplification testing on population-based incidence rates of Clostridium difficile infection. Clin Infect Dis 57:1304–1307. https://doi.org/10.1093/cid/cit492.
9. Riggs MM, Sethi AK, Zabarsky TF, Eckstein EC, Jump RL, Donskey CJ. 2007. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic Clostridium difficile strains among long-term care facility residents. Clin Infect Dis 45:992–998. https://doi.org/10.1086/521854.

10. Galdys AL, Nelson JS, Shutt KA, Schlackman JL, Pakstis DL, Pasculle AW, Marsh JW, Harrison LH, Curry SR. 2014. Prevalence and duration of asymptomatic Clostridium difficile carriage among healthy subjects in Pittsburgh, Pennsylvania. J Clin Microbiol 52:2406–2409. https://doi.org/10.1128/JCM.00222-14.
11. Dubberke ER, Han Z, Bobo L, Hink T, Lawrence B, Copper S, Hoppe-Bauer J, Burnham CA, Dunne WM, Jr. 2011. Impact of clinical symptoms on interpretation of diagnostic assays for Clostridium difficile infections. J Clin Microbiol 49:2887–2893. https://doi.org/10.1128/JCM.00891-11.
12. Burnham CA, Carroll KC. 2013. Diagnosis of Clostridium difficile infection: an ongoing conundrum for clinicians and for clinical laboratories. Clin Microbiol Rev 26:604–630. https://doi.org/10.1128/CMR.00016-13.
13. Tille P. 2017. Bailey and Scott's diagnostic microbiology, 14th ed. Elsevier Health Sciences, Inc., St. Louis, MO.
14. Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang YW, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH. 2015. Overdiagnosis of Clostridium difficile infection in the molecular test era. JAMA Intern Med 175:1792–1801. https://doi.org/10.1001/jamainternmed.2015.4114.
15. U.S. Food and Drug Administration. 2017. Nucleic acid-based tests. U.S. Food and Drug Administration, Silver Spring, MD. https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm. Accessed 3/7/2018.
16. Koo HL, Van JN, Zhao M, Ye X, Revell PA, Jiang ZD, Grimes CZ, Koo DC, Lasco T, Kozinetz CA, Garey KW, DuPont HL. 2014. Real-time polymerase chain reaction detection of asymptomatic Clostridium difficile colonization and rising C. difficile-associated disease rates. Infect Control Hosp Epidemiol 35:667–673. https://doi.org/10.1086/676433.
17. Becton, Dickinson, and Company. BD MAX Cdiff package insert. Becton, Dickinson, and Company, Sparks, MD. http://www.bd.com/resource.aspx?IDX=27202.
18. Meridian Bioscience, Inc. 2016. ImmunoCard toxins A & B package insert. Meridian Bioscience, Inc., Cincinnati, OH. http://www.meridianbioscience.com/Content/Assets/PackInsert/8_5x11_CLEAN_SN11223_ICTAB_PI_REV_12-16.pdf.
19. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, Dubberke ER, Garey KW, Gould CV, Kelly C, Loo V, Shaklee Sammons J, Sandora TJ, Wilcox MH. 2018. 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 66:e1–e48. https://doi.org/10.1093/cid/cix1085.
20. Charlson ME, Pompei P, Ales KL, MacKenzie CR. 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 40:373–383. https://doi.org/10.1016/0021-9681(87)90171-8.
21. Truong CY, Gombar S, Wilson R, Sundararajan G, Tekic N, Holubar M, Shepard J, Madison A, Tompkins L, Shah N, Deresinski S, Schroeder LF, Banaei N. 2017. Real-time electronic tracking of diarrheal episodes and laxative therapy enables verification of Clostridium difficile clinical testing criteria and reduction of Clostridium difficile infection rates. J Clin Microbiol 55:1276–1284. https://doi.org/10.1128/JCM.02319-16.
22. Peterson LR, Mehta MS, Patel PA, Hacek DM, Harazin M, Nagwekar PP, Thomson JRB, Robicsek A. 2011. Laboratory testing for Clostridium difficile infection: light at the end of the tunnel. Am J Clin Pathol 136:372–380. https://doi.org/10.1309/AJCPTP5XKRSNXVIL.
23. Senok AC, Aldosari KM, Alowaisheq RA, Abid OA, Alsuhaibani KA, Khan MA, Somily AM. 2017. Detection of Clostridium difficile antigen and toxin in stool specimens: comparison of the C. difficile Quik Chek Complete enzyme immunoassay and GeneXpert C. difficile polymerase chain reaction assay. Saudi J Gastroenterol 23:259–262. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5539681/.
24. Jamal W, Pauline EM, Rotimi VO. 2014. Comparative performance of the GeneXpert C. difficile PCR assay and C. diff Quik Chek Complete kit assay for detection of Clostridium difficile antigen and toxins in symptomatic community-onset infections. Int J Infect Dis 29:244–248. https://doi.org/10.1016/j.ijid.2014.10.025.
25. Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH, Society for Healthcare Epidemiology of America, Infectious Diseases Society of America. 2010. 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 31:431–455. https://doi.org/10.1086/651706.
26. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. 2013. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol 108:478–498. https://doi.org/10.1038/ajg.2013.4.