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RESEARCH

Evaluation of different algorithm schemes in the laboratory diagnosis of Clostridioides difficile

Clostridioides difficile'nin laboratuvar tanısında farklı algoritma şemalarının değerlendirilmesi

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Abstract

Purpose: *Clostridioides difficile* infection is a major cause of antibiotic-associated diarrhea, particularly in healthcare settings. This study aims to evaluate the applicability, speed, cost-effectiveness, and diagnostic accuracy of different laboratory algorithm schemes for *C. difficile* infection in a routine clinical setting.

Materials and Methods: A total of 479 stool samples from patients suspected of having *C. difficile* infection were analyzed using glutamate dehydrogenase enzyme immunoassay, toxin A/B enzyme immunoassay, toxigenic culture, and real-time polymerase chain reaction. The sensitivity, cost-effectiveness and overall diagnostic accuracy of these methods were assessed when applied in different algorithmic sequences.

Results: Of the 479 samples, 52 were positive for glutamate dehydrogenase antigen. Polymerase chain reaction exhibited the highest sensitivity, detecting *C. difficile* in 55.8% of glutamate dehydrogenase -positive samples, followed by toxigenic culture at 25.0%, and toxin A/B enzyme immunoassay at 23.1%. The combination of glutamate dehydrogenase screening followed by polymerase chain reaction was the most effective diagnostic approach, offering both high sensitivity and cost-effectiveness.

Conclusion: The study emphasizes the importance of a multi-step diagnostic algorithm, particularly starting with glutamate dehydrogenase screening followed by PCR, to improve the accuracy and cost-effectiveness of *C. difficile* infection diagnosis. These findings support the need for tailored diagnostic strategies based on laboratory resources and patient population characteristics.

Keywords:. Clostridioides difficile, diagnostic algorithm, glutamate dehydrogenase screening, PCR, toxigenic culture, enzyme immunoassay

Öz

Amaç: *Clostridioides difficile* enfeksiyonu, başlıca hastanelerde olmak üzere, antibiyotiğe bağlı ishalin önemli bir nedenidir. Bu çalışmanın amacı, *C. difficile* enfeksiyonu için rutin klinik pratikte farklı laboratuvar algoritma şemalarının uygulanabilirliğini, hızını, maliyet etkinliğini ve tanısal doğruluğunu değerlendirmektir.

Gereç ve Yöntem: *C. difficile* enfeksiyonu şüphesi olan hastalardan alınan toplam 479 dışkı örneği, glutamat dehidrogenaz enzim immunoassay, toksin A/B immunoassay, toksijenik kültür ve gerçek zamanlı polimeraz zincir reaksiyonu kullanılarak incelenmiştir. Bu yöntemlerin farklı algoritmik dizilimlerde uygulandığında duyarlılıkları, maliyet etkinlikleri ve tanısal doğrulukları değerlendirilmiştir.

Bulgular: 479 örnekten 52'sinde glutamat dehidrogenaz antijeni pozitif bulunmuştur. Polimeraz zincir reaksiyonu, glutamat dehidrogenaz pozitif örneklerin %55,8'inde *C. difficile* saptayarak en yüksek duyarlılığı göstermiştir; bunu %25 ile toksijenik kültür ve %23,1 ile toksin A/B enzim immunoassay izlemiştir. Glutamat dehidrogenaz antijeni taraması ve ardından yapılan polimeraz zincir reaksiyonundan oluşan algoritma hem yüksek duyarlılık hem de maliyet etkinliği sunan en etkin tanısal yaklaşım olmuştur.

Sonuç: Bu çalışma, *C. difficile* enfeksiyonu tanısında, başta glutamat dehidrogenaz antijen taraması ile başlayan ve ardından polimeraz zincir reaksiyonu uygulanan olmak üzere çok aşamalı algoritmaların tanısal doğruluğu ve maliyet etkinliği artırmadaki önemini vurgulamaktadır. Bu bulgular, laboratuvar kaynakları ve hasta popülasyonunun özelliklerine dayalı olarak uyarlanaan tanı stratejilerin gerekliliğini desteklemektedir.

Anahtar kelimeler: Clostridioides difficile, tanı algoritması, glutamat dehidrogenaz taraması, PCR, toksijenik kültür, enzim immunassay

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INTRODUCTION

Clostridioides difficile (formerly *Clostridium difficile*) is a Gram-positive, spore-forming, obligate anaerobic bacillus and is recognized as one of the leading causes of antibiotic-associated diarrhea (AAD) and colitis. This bacterium, primarily pathogenic through its toxins, poses a significant threat, particularly in nosocomial infection contexts. It is estimated that approximately 25% of all AAD cases are caused by *C. difficile* infection (CDI)[1]. CDI manifests a wide variety of clinical presentations, ranging from asymptomatic colonization to severe gastrointestinal conditions such as toxic megacolon, bowel perforation, and pseudomembranous colitis (PMC). The inappropriate and extensive use of antibiotics plays a critical role in the emergence of these clinical conditions[2, 3]. Although antibiotics can stop the growth of or kill pathogenic bacteria, they often disrupt the normal flora, predisposing the host to secondary infections[4]. *C. difficile* is thought to be responsible for nearly all severe cases and PMC[1, 5].

Advances in medical science and technology have significantly improved the diagnosis and treatment of severe diseases. However, despite the development of sensitive diagnostic methods, effective antibiotic treatments, and hospital infection control measures, CDI continues to be a significant health problem. The increasing interest in CDI has led to the reevaluation of diagnostic methods used for the etiological diagnosis of the infection. While several different algorithmic approaches have been proposed for CDI diagnosis, the most effective diagnostic strategy remains a topic of debate[6]. In Turkiye, most diagnostic and surveillance studies have relied on immunochromatographic tests and/or enzyme immunoassay (EIA) methods, which are no longer recommended as standalone diagnostic tools[7, 8].

This study addresses a gap in the literature by evaluating the effectiveness of different diagnostic algorithms for *C. difficile* infection in routine clinical settings, particularly in Turkiye. Diagnostic methods such as glutamate dehydrogenase enzyme immunoassay (GDH-EIA), toxin A/B EIA, toxigenic culture, and real-time polymerase chain reaction (PCR) are assessed based on turnaround time, cost-effectiveness, and diagnostic accuracy to identify the most suitable algorithm for adaptation in various laboratory environments. The hypothesis is that a multistep approach, particularly the combination of glutamate dehydrogenase screening followed by PCR, offers superior sensitivity and cost-effectiveness.

MATERIALS AND METHODS

Study design and sample

This study included 479 stool samples collected from patients older than two years who were clinically suspected to have CDI. All samples were stored and processed under standardized laboratory conditions at the Bacteriology Laboratory of Istanbul University Faculty of Medicine. The laboratory adheres to standardized diagnostic protocols, with all procedures performed by qualified personnel, ensuring the integrity of samples and the reliability of patient records through strict adherence to clinical standards. The study was approved by the Clinical Intervention Ethics Committee of Istanbul University Faculty of Medicine (1651/20.11.2012).

Only watery or semi-solid stool samples were included, while formed or solid samples were excluded. Duplicate or multiple samples from the same patient, as well as those with incomplete clinical data, insufficient stool samples for diagnostic testing, or those who had recently received CDI treatment, were excluded.

Diagnostic tests

GDH EIA

All stool samples were initially screened for the presence of glutamate dehydrogenase (GDH) using the Ridascreen® Clostridium difficile GDH EIA kit (R-Biopharm AG, Germany). This antigen test served as the initial step to identify potential CDI.

Following the GDH screening, three additional tests were performed concurrently on the positive samples to confirm the diagnosis of *C. difficile,* and to compare their effectiveness.

Toxin A/B EIA

The Ridascreen® Clostridium difficile Toxin A/B EIA kit (R-Biopharm AG, Germany) was used to detect toxins A and B directly in the stool samples.

Toxigenic culture

Samples were cultured on cycloserine-cefoxitin fructose agar [CCFA (Oxoid, UK)] and blood agar, and the media were incubated under anaerobic

conditions for 48 hours to isolate *C. difficile*. The presence of characteristic colonies, such as yellow, 2-3 mm diameter S-type colonies on CCFA or irregular, 3-5 mm diameter, non-hemolytic, white, frosted-glass-like colonies on blood agar, was confirmed using standard microbiological techniques, including Gram staining and controlled culture under both aerobic and anaerobic conditions. The presence of the characteristic barnyard odor in the culture medium was also noted as part of the differential diagnosis. Following the isolation, the *C. difficile* isolates were tested for toxin production using the Toxin A/B detection kit (R-Biopharm AG, Germany).

Real-Time PCR:

The GeneXpert *C. difficile* system (Cepheid, USA) was employed to detect Tox B, binary toxin and tcdC deletion nt 117 to identify hypervirulent strain 027/NAP1/BI.

Statistical analysis

The sample size was determined based on an estimated CDI prevalence of 10% in the hospital population. A power analysis was conducted to ensure that the sample size would provide 80% power to detect statistically significant differences between diagnostic methods with a 95% confidence level.

All data obtained from the diagnostic tests were analyzed using SPSS version 17.0 statistical software. Comparative analyses were performed using the Chi-square test to assess the relationship between categorical variables, and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) calculated to evaluate the performance of the diagnostic methods. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 479 patients consisting of 216 (45.1%) females and 263 (54.9%) males were included. Of these, 287 (59.9%) were hospitalized, and 192 (40.1%) were outpatients. The majority of hospitalized patients were from internal medicine wards (39.2%), followed by transplantation/hematopoietic stem cell units (8.1%), surgical wards (6.5%), intensive care units (3.3%), and pediatric wards (2.7%).

GDH antigen was found to be positive in 52 (10.9%) of 479 stool samples. Out of the 52 GDH-positive samples, 13 (25.0%) were positive by toxigenic culture, 12 (23.1%) were positive by toxin A/B EIA and 29 (55.8%) were positive by PCR.

Table 1. Demographic and clinical characteristics of all patients and those with positive test results

Characteristic	All Patients (n=479)	GDH Positive (n=52)	Toxigenic Culture Positive (n=13)	Toxin A/B EIA Positive (n=12)	PCR Positive (n=29)
Female	216 (45.1%)	23 (44.2%)	7 (53.8%)	5 (41.7%)	14 (48.3%)
Male	263 (54.9%)	29 (55.8%)	6 (46.2%)	7 (58.3%)	15 (51.7%)
Hospitalized	287 (59.9%)	33 (63.5%)	11 (84.6%)	8 (66.7%)	27 (93.1%)
Outpatient	192 (40.1%)	19 (36.5%)	2 (15.4%)	4 (33.3%)	2 (6.9%)
Age 2-18 years	69 (14.4%)	5 (9.6%)	1 (7.7%)	2 (16.7%)	5 (17.2%)
Age 19-64 years	321 (67.0%)	34 (65.4%)	9 (69.2%)	7 (58.3%)	17 (58.6%)
Age >65 years	89 (18.6%)	13 (25.0%)	3 (23.1%)	3 (25.0%)	7 (24.1%)
Watery or mucoid stool	286 (59.7%)	40 (76.9%)	10 (76.9%)	8 (66.7%)	22 (75.9%)
Semi-formed stool	193 (40.3%)	12 (23.1%)	3 (23.1%)	4 (33.3%)	7 (24.1%)
Antibiotic use	295 (61.6%)	47 (90.4%)	12 (92.3%)	11 (91.7%)	26 (89.7%)
Underlying disease	418 (87.3%)	45 (86.5%)	11 (84.6%)	10 (83.3%)	25 (93.1%)

GDH: glutamate dehydrogenase; PCR: polymerase chain reaction; EIA: enzyme immunoassay.

The study found that when toxigenic culture was considered the gold standard, PCR demonstrated the highest sensitivity (100%) and NPV (100%) for detecting *C. difficile* in GDH-positive samples, followed by toxin A/B EIA with a sensitivity of 54% and an NPV of 87%. No cases were found where toxigenic culture was positive, but PCR was negative. Specificity and PPV are not provided in this analysis,

as toxigenic culture may miss positive cases, potentially underestimating the true performance of these diagnostic methods.

The demographic and clinical characteristics of all patients, as well as those with positive test results, are summarized in the Table. Further statistical analysis revealed no significant difference in CDI positivity across different age groups ($p>0.05$). In contrast, hospitalized patients exhibited a significantly higher likelihood of CDI compared to outpatients ($p<0.05$). Moreover, a strong association was observed between prior antibiotic exposure and CDI, with individuals who had received antibiotics being significantly more likely to test positive for *C. difficile* ($p<0.05$).

The average cost per sample when using GDH-EIA as the initial screening method was calculated to be approximately $3.5, taking into account the number of samples processed and the controls employed. The positivity rate observed with GDH-EIA was 10.9%. By limiting the use of the more expensive PCR kit (with a unit cost of approximately $45) to only 10.9% of the patient samples, the effective cost per PCR test was reduced to $4.9. As a result, the total cost per sample when using this two-step algorithm was $8.4.

DISCUSSION

The use of antibiotics has significantly improved the management of bacterial infections, reducing morbidity and mortality. However, this benefit comes with the cost of disrupting the normal gut microbiota, leading to infections such as CDI. CDI is now the leading cause of nosocomial diarrhea and has become the most commonly identified cause of healthcare-associated infection in adults in the United States[6]. According to a 2012 CDC report, which included data from 711 acute care hospitals across 28 states, the pooled rate of hospital-onset CDI was reported to be 7.4 per 10,000 patient-days[9]. Our study reported a CDI prevalence of 6% by the algorithm consisting of GDH and PCR, which is consistent with the findings from various studies in Turkiye, where prevalence rates have been reported to range between 2.8% and 14.3% depending on the diagnostic methods used[7,8,10].

Advanced age is a well-known risk factor for CDI, with older adults being particularly more vulnerable due to changes in gut flora, a decline in immune function, and the increased likelihood of comorbidities[1,11]. Loo et al. found that age is a significant risk factor, with the risk of CDI increasing approximately by 2% for each additional year of age after 18[12]. However in our study, the age distribution among positive cases for toxigenic culture, PCR, and toxin A/B EIA did not show significant deviation from the overall distribution. It should be noted that the presence of positive cases in the >65 years group across all tests underscores the heightened vulnerability of older adults to severe outcomes[6]. Furthermore, underlying conditions were present in 25 (86.2%) out of 29 of our PCR-positive CDI patients, with hematologic malignancies found in 10 out of 29 (34.5%). This supports the findings of other studies that have identified cancer and the use of chemotherapeutics as significant risk factors for CDI[6,13].

Antibiotic use remains the most significant risk factor for CDI, as well-documented in the literature. In our study, nearly 90% of patients with positive test results across all diagnostic methods had a history of antibiotic use, with beta-lactam antibiotics being the most prescribed. This is consistent with the findings of Slimings et al., who demonstrated that beta-lactam antibiotics such as carbapenems and third and fourth generation cephalosporins, exhibit the strongest association with healthcare-associated CDI[14]. Likewise, Karp et al. reported that cephalosporins are associated with a more rapid onset and a higher incidence of CDI compared to other antibiotic classes during active treatment[15]. These findings support the well-established association between antibiotic use and the risk of developing CDI and underscore the importance of antibiotic stewardship programs aimed at reducing the unnecessary use of high-risk antibiotics to prevent CDI.

The duration of hospital stay is directly correlated with the risk of acquiring CDI, as it likely reflects greater exposure to the bacterium, increased antibiotic use, and the severity of underlying illness[6]. In our study, hospitalized patients had a significantly higher rate of positive results across all tests, particularly in PCR and toxigenic culture, indicating a greater likelihood of CDI among inpatients. Notably, the toxin A/B EIA test in our study appears to have missed some cases of *C. difficile* in hospitalized patients, potentially underestimating the true prevalence of CDI in the vulnerable population.

Diagnosing CDI remains challenging due to the lack of a universally accepted "gold standard" test. In our study, we employed different diagnostic methods, including GDH as a screening test followed by

toxigenic culture, toxin A/B EIA, and real-time PCR. PCR detected *C. difficile* in 29 patients, while toxigenic culture confirmed the presence of the bacterium in 13 cases. This discrepancy highlights the high sensitivity of PCR but also raises concerns about its specificity as it may detect colonization rather than the active infection[16,17]. This underscores the importance of careful clinical evaluation when requesting the tests and selecting the samples. Although other etiologies for diarrhea are possible, the likelihood of CDI is substantially elevated when the patient presents with diarrheal symptoms. The challenges associated with toxigenic culture, such as the need for strict anaerobic conditions and the longer turnaround time required for results, were also observed in our study. Toxigenic culture is often regarded as a reference method; however, its applicability in routine diagnostics is limited because it is laborious and time-consuming. Toxin A/B EIA, though easier with shorter turnaround time than toxigenic culture, demonstrated lower sensitivity, detecting CDI in only 12 of the cases. This finding is consistent with the findings of various other studies, which suggest that while toxin A/B EIA is more practical for routine diagnostics, it often misses cases that PCR or toxigenic culture would detect. On the other hand, Wilcox et al. noted that while toxigenic culture is accurate, it is not feasible for routine use due to its complexity and the longer turnaround time, and that toxin A/B EIA, despite its ease of use, is not accurate enough as a standalone diagnostic tool due to its lower sensitivity[17].

Given the limitations of individual diagnostic tests, recent guidelines recommend using diagnostic algorithms that combine multiple methods to improve the accuracy[6]. The multistep method has proven both efficient and cost-effective reducing the per-sample cost from $45 to $8.4 in our study. This aligns with findings of Goldenberg et al. and Vasoo et al., who demonstrated that such an approach enhances diagnostic accuracy while controlling the costs[18, 19]. Similarly, Cançardo et al. found that a two-step algorithm is more cost-effective compared to stand-alone NAAT (nucleic acid amplification test) in developing countries[20]. In our study, we concluded that an algorithm incorporating GDH screening followed by PCR is the most effective approach for diagnosing CDI. Such an algorithm allows the more expensive PCR tests to be reserved for cases whereas initial screening by GDH indicates a potential infection, thereby optimizing resource use. Comparative analysis with other studies further supports the effectiveness of such a diagnostic approach that includes the use of PCR as a secondary and confirmative test[20]. For instance, in a study conducted by Novak-Weekley et al. it was reported that the sensitivity and negative predictive value of real-time PCR were superior to those of other diagnostic methods, including EIA and toxigenic culture[21].

The laboratory results for CDI should be interpreted with caution, and each laboratory should establish the most suitable diagnostic algorithm according to its capacity and resources. We believe that GDH screening which is reported to have a negative predictive value of >99%[18], can help reduce unnecessary testing, but in cases where the number of tests is limited, laboratories might consider using PCR as the primary diagnostic method. However, as mentioned earlier, the challenges in distinguishing between the colonization and active infection should be kept in mind. Moreover, incorporating toxin A/B EIA as a part of third step can be considered to enhance the specificity of the algorithm in the diagnosis.

Although this study provides valuable insights into the diagnosis and epidemiology of CDI, several limitations should be acknowledged. First, the study was conducted at a single institution, which may limit the generalizability of the findings to other settings with different patient populations and diagnostic practices. Additionally, the study did not include a comprehensive comparison of all available diagnostic methods and algorithms, which could provide further context on the efficacy of the diagnostic approaches used. Finally, the nature of the study and the reliance on clinical records may introduce bias, particularly in the assessment of patient symptoms and outcomes. These limitations suggest the need for further multicenter, prospective studies to validate and expand upon our findings.

In conclusion, the present study reinforces the importance of using a comprehensive diagnostic approach to detect CDI effectively. The combination of GDH screening and PCR as a part of a two-step algorithm offers a practical, accurate, and cost-effective method for diagnosing CDI. The findings from this study underscore the need for further research to enhance the understanding and diagnosis of CDI. Future studies should focus on multicenter investigations to validate the diagnostic algorithms across diverse patient populations and healthcare settings.

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REFERENCES

1. Gerding DN, Young VB, Donskey CJ. Clostridioides difficile (Formerly Clostridium difficile) Infection. In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 9th ed (Eds JE Bennett, R Dolin, MJ Blaser):2933-47. Philadelphia, Elsevier, 2020.
2. Bartlett JG, Chang TW, Gurwith M, Gorbach SL, Onderdonk AB. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. N Engl J Med. 1978;298:531-4.
3. Kelly CP, LaMont JT. Clostridium difficile more difficult than ever. N Engl J Med. 2008;359:1932-40.
4. Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol. 2009;7:526-36.
5. Martinez FJ, Leffler DA, Kelly CP. Clostridium difficile outbreaks: prevention and treatment strategies. Risk Manag Healthc Policy. 2012;5:55-64.
6. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE 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.
7. Öksüz Ş, Danış A, Öztürk E, Çalışkan E, Akar NK, Sungur MA. Uzun süre hastanede yatan hastalarda Clostridium difficile kolonizasyonunun araştırılması. Anadolu Kliniği Tıp Bilimleri Dergisi. 2017;22:177-82.
8. İris NE, Demirel A, Koçulu S, Çevik E, Ordu A, Küçükkaya RD. The evaluation of Clostridium difficile toxin A ve B positivity rates and epidemiological characteristics in febrile neutropenic patients with diarrhea. Mediterr J Infect Microb Antimicrob. 2015;4:14.
9. McDonald LC, Lessa F, Sievert D, Wise M, Herrera R, Gould C et al. Vital signs: preventing Clostridium difficile infections. MMWR Morb Mortal Wkly Rep. 2012;61:157-62.
10. Altindiş M, Usluer S, Ciftci H, Tunç N, Cetinkaya Z, Aktepe OC. Investigation of the presence of Clostridium difficile in antibiotic associated diarrhea patients by culture and toxin detection methods. Mikrobiyol Bul. 2007;41:29-37.
11. Rao K, Micic D, Chenoweth E, Deng L, Galecki AT, Ring C et al. Poor functional status as a risk factor for severe Clostridium difficile infection in hospitalized older adults. J Am Geriatr Soc. 2013;61:1738-42.
12. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N et al. Host and pathogen factors for Clostridium difficile infection and colonization. N Engl J Med. 2011;365:1693-703.
13. Buchner AM, Sonnenberg A. Medical diagnoses and procedures associated with Clostridium difficile colitis. Am J Gastroenterol. 2001;96:766-72.
14. Slimings C, Riley TV. Antibiotics and healthcare facility-associated Clostridioides difficile infection: systematic review and meta-analysis 2020 update. J Antimicrob Chemother. 2021;76:1676-88.
15. Karp J, Edman-Wallér J, Jacobsson G. Duration from start of antibiotic exposure to onset of Clostridioides difficile infection for different antibiotics in a non-outbreak setting. Infect Dis (Lond). 2024; doi:10.1080/23744235.2024.2375602.
16. Crobach MJT, Vernon JJ, Loo VG, Kong LYP, Péchiné S, Wilcox MH et al. Understanding Clostridium difficile colonization. Clin Microbiol Rev. 2018;31:e00021-17.
17. Wilcox MH, Planche T, Fang FC, Gilligan P. What is the current role of algorithmic approaches for diagnosis of Clostridium difficile infection? J Clin Microbiol. 2010;48:4347-53.
18. Goldenberg SD, Cliff PR, Smith S, Milner M, French GL. Two-step glutamate dehydrogenase antigen real-time polymerase chain reaction assay for detection of toxigenic Clostridium difficile. J Hosp Infect. 2010;74:48-54.
19. Vasoo S, Stevens J, Portillo L, Barza R, Schejbal D, Wu MM et al. Cost-effectiveness of a modified two-step algorithm using a combined glutamate dehydrogenase/toxin enzyme immunoassay and real-time PCR for the diagnosis of Clostridium difficile infection. J Microbiol Immunol Infect. 2014;47:75-8.
20. Cançado GGL, Abreu ES, Nardelli MJ, Serwa P, Brachmann M. A cost of illness comparison for toxigenic Clostridioides difficile diagnosis algorithms in developing countries. Anaerobe. 2021;70:102390
21. Novak-Weekley SM, Marlowe EM, Miller JM, Cumpio J, Nomura JH, Vance PH, et al. Clostridium difficile testing in the clinical laboratory by use of multiple testing algorithms. J Clin Microbiol. 2010;48:889-93.