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Original Article

The positivity rates of a novel test in the patients with suspected clostridioides difficile associated diarrhea

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Abstract

Aim: To evaluate the positivity rates of Clostridioides difficile infection (CDI) in the stool samples with a novel test and clinical features of positive cases.

Material and Methods: The frequency of C. difficile in a total of 654 stool samples were examined with the BD Max Cdiff Test (Becton Dickinson, USA) between January 2014 and June 2019, and the clinical/demographic characteristics of the positive cases were evaluated in a university hospital.

Results: A total 56 (8.56%) samples belonging to 49 cases aged 3-84 year were determined as positive for CDI among total 654 stool samples. Forty-one (89.1%) out of 46 positive cases whose clinical reports were available had a history of hospitalization in the last three months with an average 14.9 days, and 39 patients (84.7%) received antimicrobial treatment in the last three months for an average 12.2 days. It was observed that 40 (86.9%) out of 46 positive cases had at least one underlying chronic disease; and 38 (82.6%) patients used anti-acid agents.

Conclusion: In this study, although the risk factors similar to those reported in the international literature were also found for our patients, the incidence of CDI was found to be lower than that reported worldwide. In this context, it is required that the patients in the risk group for CDI must be identified well, and correct and fast methods should be used for diagnosing the infection.

Keywords: Clostridioides difficile; risk factors; antibiotic-associated diarrhea

INTRODUCTION

Clostridioides (formerly known as Clostridium) difficile, which is an anaerobic, gram-positive, spore-forming bacillus, is highly infectious; and is the most common reason of antibiotic-related nosocomial diarrhea (1). Clostridioides difficile infection (CDI), which may vary from mild diarrhea that has no complications to fatal toxic megacolon and toxic pseudomembranous colitis, might cause large epidemics. For this reason, infection control measures like contact isolation must be applied to these patients; and antimicrobial treatment should be initiated. CDI must be considered in diarrhea patients who have risk factors like hospitalization, advanced age, and antibiotic use (1,2). However, since other bacterial and viral infections, and chemicals may also cause diarrhea, detecting the main virulence factor (Toxin A (TcdA) and/or toxin B (TcdB)) mediating CDI, and thus, differentiating the toxigenic isolates from the none-toxin-producing isolate is important in the diagnosis and treatment (2).

It is fundamental to show the presence of free toxin in the stool or toxigenic C. difficile species in laboratory diagnosis. The toxicogenic culture and fecal cytotoxicity tests that are employed for this purpose are accepted as the reference methods; however, they cannot be used as a routine diagnosis in most laboratories since both methods are difficult and time-consuming (3). The existing Enzyme Immunoassay (EIA) methods that are employed for rapid diagnosis of Toxin A and/or B and C. difficile-specific glutamate dehydrogenase (GDH) enzyme detection are fast and specific; however, it was also reported that their sensitivity and clinical specificity are low (4). The Nucleic Acid Amplification Tests (NAAT) that are used in our present day are costly but fast and highly sensitive methods, which can minimize the disputes between

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the laboratory and the clinic. In actual fact, because of the limitations in one or more of the current methods, international guidelines recommend the use of various multistage test algorithms like EIA tests for detection of GDH and toxin or NAATs alone in case of toxin detection with NAAT or acceptance of CDI-specific aqueous stools for the diagnosis of CDI provided that they are confirmed by NAATs (1-4).

The nucleic acid-based diagnostic tests that were developed commercially for the detection of toxigenic C. difficile were approved by the US Food and Drug Administration (FDA) in 2008 for the first time; and today, their different commercial forms of sensitivity and specificity close to the toxicogenic culture are used in laboratories (3). Among these, BD Max Cdiff platform (Becton Dickinson, USA), which received FDA approval in 2013 is a fully-automated system that can detect the C. difficile TcdB gene with extremely high sensitivity and specificity within approximately 100-120 minutes directly from fecal samples with real-time polymerase chain reaction. The sensitivity, specificity, positive- and negative-predictive values were reported to be 97.7%, 99.7%, 97.7% and 99.7%, respectively (5). In this study, the purpose was to examine the positivity rates that were detected with the BD Max Cdiff Test, and the clinical/demographical characteristics of patients with positivity in stool samples that were sent to our laboratory with CDI suspicion.

MATERIAL and METHODS

In this study, the positivity rates of the C. difficile TcdB gene in stool samples sent to Inonu University Faculty of Medicine Molecular Microbiology Laboratory between January 2014 and June 2019 from various clinics were analyzed, retrospectively. To examine a total of 654 stool samples for C. difficile, real-time PCR-based BD Max Cdiff Test (BD Diagnostics, Sparks, MD) was used. Following the loading of the samples to the automated platform in line with the recommendations of the manufacturer, the DNA extraction and amplification was carried out by the BD Max System; and the results were reported as C. difficile positive or negative.

The laboratory (direct stool microscopy and stool culture), demographical (years, ages, genders, clinical/polyclinic manifestations) and clinical (antibiotic / antacid / chemotherapeutic drug use, underlying disease status) data of the patients who were reported to be Toxin B positive were analyzed retrospectively in the hospital automation system.

In the routine bacteriology laboratory examinations of the stool samples, the presence of erythrocyte/leukocyte were evaluated microscopically, and fecal culture was performed for pathogenic bacteria such as Salmonella and Shigella. The samples were cultured on blood agar, Eosin-Methylene Blue (EMB) and Salmonella-Shigella agar media.

For each patient, the data in the last three months were evaluated for the hospitalization and antimicrobial treatment history, which were previously reported the most important risk factors among the patients.

RESULTS

A total 654 stool samples were processed for CDI during the study period. The TcdB gene of 56 (8.56%) out of the 654 stool samples, which were accepted as suitable for BD Max Cdiff Test, was found to be positive. It was determined that the ages of these positive patients (n=49) were between 3-83 years, and the demographic data of these cases were evaluated (Table 1). When the routine stool culture results of the positive cases were examined, it was determined that there were no Salmonella/Shigella growth in the culture. In direct microscopic examination, leukocytes and/or erythrocytes were not detected in 60% of the positive cases, and abundant amounts of leukocytes were detected in 25% of the cases. It was determined that 60% of the toxin gene positive samples belonged to inpatients who were hospitalized in the Departments of Infectious Diseases (32.35%), Pediatric Gastroenterology (17.65%), Organ Transplantation (14.70%), and various intensive care units (11.76%).

Table 1. Laboratory and demographic data of positive patients

Patient Characteristics	BDmax Cdiff Positive Patient % (n=49)
Average Age	41.92
> 65 years of age	20.40
Gender	
Female	48.98
Male	51.02
Polyclinic	30.61
Clinic	60.39
Fecal leukocytes-positive stools	40
Fecal erythrocytes-positive stools	15

Since the clinical reports of three out of the 49 positive patients were not available in the system, the clinical data of these three patients could not be examined, and the clinical data evaluation was made over 46 patients (Table 2). It was determined that 89.13% of these 46 cases had a history of hospitalization in the last three months; and the mean duration of hospitalization was 14.97 days. It was seen that 84.78% of the patients were treated with any antibiotics in the last three months; and the average duration of antibiotic therapy was 12.23 days.

Table 2. Risk factor distribution of positive patients

Risk Factors	BDmax Cdiff Positive Patient % (n=46)
Last three months of hospitalization	89.13
> 10 days of hospitalization	70.73
Presence of underlying disease	86.94
Solid organ transplantation	19.56
Cancer	13.04
Chronic renal failure	13.04
Inflammatory Bowel Disease	23.91
Gastrointestinal system operation	17.39
Drug use in the last three months	
Antibiotic exposure	84.78
Carbapenem	38.46
β-lactam/ β-lactamase inhibitor combination	28.20
Third-generation cephalosporin	25.64
Fluoroquinolone	23.07
Glycopeptide, Tetracycline, Macrolide	2.56
Multiple antibiotherapy	20.51
Use of any anti-acid agent (proton pump inhibitor/histamine-2 receptor blocker)	82.60
Use of antineoplastic agent	15.21
Use of immunosuppressive agents	34.78

DISCUSSION

There are limited data on the prevalence of C. difficile in Turkey. According to the results of some studies in which mostly EIA methods were used, the prevalence of C. difficile varies between 4.3-24% in our country (6-8). Of course, the differences between the study results stem from the diagnostic tests employed being closely related to the presence of risk factors in the cases included in the studies, the number of the clinical samples diagnosed correctly, the changes in the conditions and duration of the transport of the samples, and the differences in hospital antibiotic use policies. In this study, the C. difficile positivity was found to be 8.56% in approximately five years period in our hospital. A commercially-available EIA-based test was also routinely used in our hospital for approximately one year within the study period, and then the BDmax Cdiff test alone was continued to be used. For this reason, it was not possible to make a pre-elimination like the routinely sent stool samples being mucus and aqueous during sample acceptance; and all samples requested for C. difficile were examined without the quality of the clinical samples was evaluated. This situation most likely caused relatively lower positivity rates for CDI, by increasing the sample size. In addition, it was determined in the studies involving certain risk groups that sample quality can be evaluated and especially in studies in which the sample quality might be evaluated by prospective planning, positivity rates were reported to be higher. In the study conducted by Ünlü et al. (9) in which the distribution of gastroenteritis agents was examined retrospectively in our country for approximately one year, the C. difficile positivity was reported to be 7.4% in all agents; however, positivity was reported as 4.3% in another study in which the patients did not have any hospitalization history but had a history of using antibiotics in the last three months. It was emphasized in the study that this proportional decrease might be due to the low sensitivity of the method, especially because of the improper stool transport conditions and duration, which are the main problems especially in EIA tests, or due to early empirical treatment (6).

Since advanced age is a potential carrier for the severity of the disease and comorbidities, it is one of the most important risk factors for CDI, as it is the case in the length of hospitalization. The daily increase in the risk of acquiring C. difficile during hospitalization represents the increase in the risk of exposure to organisms during hospitalization, exposure to antibiotics, and increase in the severity of the underlying disease (10,11). In recent years, increases were reported in CDI in pediatric patients

in whom toxigenic and/or nontoxigenic strains are frequently colonized (12). In our study that the pediatric patient group, which constituted 24.48% of the cases, was determined to be higher than the advanced age group in the other studies conducted in our country. In the study of Kostul et al. (8), it was reported that 12.6% of the cases were in the advanced age group (>65 years) and 88.8% were in the pediatric group. In another study, it was reported that 40% of the cases consisted of pediatric patients; and it was emphasized that these patients must be examined carefully (13).

The most important risk factors that are modifiable in terms of C. difficile infections is the exposure to antibiotics (11). The deterioration of the intestinal microbiota with antibiotics lasts longer, which increases the risk of CDI during the treatment and in three months after the discontinuation of the drugs. It was reported that the highest risk in terms of C. difficile infection (7-10 fold increase) was within one month after the antibiotic exposure. It was reported that prolonged antibiotic exposure and multiple antibiotherapy increases the risk of CDI (3,10). In addition, it was also reported that very limited exposure, like a single-dose surgical antibiotic prophylaxis, increased the C. difficile colonization and symptomatic disease risks (14). Almost every antibiotic can be associated with CDI in years; however, it is mentioned that the risk increases in some antibiotic classes especially in the 3rd and 4th generation cephalosporins, fluoroquinolones, carbapenems and clindamycin (11). It was determined in our study that 84.78% of the patients had an antibiotic treatment history; and 92.3% of them beta-lactam group (penicillin, cephalosporin and carbapenem) antibiotics. In a study conducted by Deniz et al. (13), positivity rate was found as 7.9% for C. difficile with culture, and they reported that 72% of the isolates were from toxigenic origin; however, the positivity rate decreased to 4.7% when the toxin was examined with the EIA directly from the stool. It was observed that toxin gene could be detected by using the molecular method in all strains which were determined to be toxin-positive in culture filtrates. In the same study, the beta lactam antimicrobial treatment rate was found as 77.8% in the patients in whom toxigenic isolate was detected. Similar to our study, another study, which examined the risk factors in C. difficile positive cases, the C. difficile positivity was determined as 11.4%; and the antibiotic use rate was reported as 83.3% in the group (8).

Cancer chemotherapy is another risk factor for CDI, partly because of the antimicrobial activity of some chemotherapeutic agents, and partly because of the immunosuppressive and neutropenic activities. In actual fact, it is already known that patient groups like organ transplantation and malignancy are potentially at risk due to the underlying immunosuppression, the frequency of exposure to healthcare services and antibiotics, and even due to the combination of these factors (10). It was reported in previous studies that in solid organ transplant recipients, the prevalence of CDI increased 5-fold when compared to other patients; and the risk of recurrent infection was around 20% (15,16). It was also reported that the patients with chronic kidney disease or end-stage renal disease had 2-2.5-fold increased CDI recurrence risk, a 1.5-fold increased severe disease risk, and high mortality risk (17). In a study that was conducted in our country, in patients with hematologic malignancies and solid organ tumors, the cases that developed diarrhea during febrile neutropenic period were examined in terms of C. difficile, and although all the cases had multiple risk factors like multiple antibiotic therapy, long-term hospitalization and chemotherapy together, the positivity rate was 4.6%. Although all these risk factors were present together, the reason for low positivity rate was argued to be the patients' being closely followed up because of their sensitive immune status and that the stool samples were taken at a very early stage (18). In other studies conducted in our country, it was reported that the majority of positive cases were concentrated in hematology/oncology and transplantation units that required receiving immunosuppressive therapy and using antibiotics extensively (7,8,13). It was seen that 86.94% of our positive patients had any underlying disease and almost half of them were transplantation, cancer and chronic renal failure patients. In addition, the use of anti-neoplastic or immunosuppressive agents in our positive patients was found to be 49.99% (Table 2).

A great number of studies reported that the use of acid suppressants like histamine-2 blockers and proton pump inhibitors are a significant risk factor for CDI (3). However, some well-controlled study results showed that long-term use of these agents as a barrier against pathogenic microorganisms was not a specific risk factor for CDI, and was a main risk for all non-CDI diarrhea agents as it would break the stomach acidity and lower the GIS microbial diversity (3,19). Although it was determined that 82.6% of the cases in our study used these agents; in another similar study, it was reported that 44% of the positive cases used these agents (8).

The most important limitation of the present study, which was conducted in a retrospective fashion, was that the individual epicrisis of BDmax Cdiff test negative patients could not be evaluated as our patient data system was not suitable; and for this reason, no comparisons could be made between toxin gene negative and positive cases. Moreover, as it was not a prospective study, and as there was only one diagnostic test used in the study process, it was not clarified whether samples were sent from certain clinically-suspicious patients. As an other limitation, we investigated only TcdB gene in this study. However, it has been reported that TcdB plays a key role in the pathogenesis of CDI and TcdA plays a role in enhancing the effects of TcdB. It has also been reported that TcdB is required for CDI virulence and that only a TcdA-producing strain lacks virulence (20). Therefore, detecting the TcdB gene is mostly adequate for diagnosis of CDI.

CONCLUSION

As a result, unlike the whole world, the management of the C. difficile infections, which has not yet increased at a significant level in our country, may be carried out with adequate infection control measures and proper antibiotic use policies, which is possible by detecting toxigenic strains with accurate and rapid diagnostic methods. Due to its high negative predictive value among the existing tests, NAATs seem to be the most appropriate test, which may be used alone as a screening test to rule out CDI. For this purpose, the clinician has a tremendous effort burden to evaluate risk factors of the patient and to choose the right stool.

Competing interests: The authors declare that they have no competing interest.

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