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COMPARISON OF LATEX AGGLUTINATION AND ELISA FOR DETECTION OF *CLOSTRIDIUM DIFFICILE* IN PATIENTS WITH DIARRHEA AND ASYMPTOMATIC GROUPS
DİYARELİ HASTALAR VE ASEMPTOMATİK GRUPLARDA *CLOSTRIDIUM DIFFICILE*'NİN SAPTANMASINDA LATEKS AGLÜTİNASYONU VE ELISA'NIN KARŞILAŞTIRILMASI

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ABSTRACT: In this study, stool specimens from 184 patients with diarrhea (82 females, 102 males), who also complained of other gastrointestinal symptoms and from the control group of 88 asymptomatic nondiarrheal patients (34 females, 54 males) were tested for *C. difficil* by using enzyme-linked immunosorbent assay (ELISA), latex agglutination (LA), and culture in our central laboratory of Erciyes University Gevher Nesibe Hospital. LA test showed that *C. difficil* colonization was positive in 17 of 184 stool specimens (9.2%) from patients with diarrhea examined. In the control group, three of 88 stool samples (3.4 %) were positive by LA. The specimens were also tested for presence of toxin A of the bacterium. The positive rate by ELISA was 6.5% (12 of 184) in the patients with diarrhea. But all of the 88 asymptomatic controls were found to be negative by ELISA. When ELISA toxin A assay was accepted as standard test, the sensitivity of LA test was determined to be 100%, its specifity was 97%, the predictive val of a positive test was 60%, and the predictive value of a negative test was 100%.

Key words: *C. difficil* , ELISA, LA

ÖZET: Bu çalışmada, diyare ve diğer gastrointestinal yakınmaları olan 184 hasta (82 kadın, 102 erkek) ve diyare mevcut olmayan 88 asemptomatik hastanın (34 kadın, 54 erkek) dışkı örneği Erciyes Üniv Gevher Nesibe Hastanesi Merkez Laboratuvarı'nda *C. difficil* açısından ELISA, lateks aglütinasyon (LA) ve kültür yöntemleri ile değerlendirilmiştir. LA ile 184 dışkı örneğinin 17'sinde (%9.2) *C. diffic* varlığı saptanmıştır. Kontrol grubunda yer alan 88 örneğin üçü (%3.4) LA ile pozitif sonuç vermiştir. Örnekler *C. difficil* toksin A açısından da test edilmiştir. ELISA ile pozitifl oranı %6.5 (184 örneğin 12'si) olarak bulunurken kontrol grubunda yer alan 88 asemptomatik hastada pozitiflik saptanmamıştır. ELISA toksin A testi standa bir yöntem olarak kabul edildiğinde LA testinin duyarlılığı %100, özgüllüğü %97, pozitif prediktif değeri % 60 ve negatif prediktif değeri %100 olarak bulunmuştur.

Anahtar kelimeler: *C. difficil* , ELISA, LA

INTRODUCTION

C. difficil causes many diseases in the patients' gastrointestinal system ranging from acute diarrhea to pseoudo-membranous enterocolitis occurring as a result of using antibiotics (1). *C. difficil* is not normally found in the human gastrointestinal flora, but it is capab colonizing asymptomatic newborns at a rate of 25-70% (2). This rate decreases to 4% at two years of age and further decreases with age. It has been reported that this rate becomes as low as 1-3% in asymptomatic adults (3-5). Although colitis associated with *C. diffici* often appears 5-10 days after antibiotics administration, it may develop with the first dose of the treatment 7). It has been reported that antineoplastic and antiviral drugs as well as bactericidal agents also cause diarrhea associated with *C. diffic* (8, 9). Among the principal methods for the diagnosis of *C. difficil* today are anaerobic culture, cell culture (for toxin B), enzyme-linked immunosorbent assay (ELISA) (for toxin A), latex agglutination (LA), and immunofluorescent antibody (I tests (10-12). In addition, it has been reported that polymerase chain reaction (PCR) is a sensitive method that differentiates *C. difficil* in stool samples from other microbiota and toxigenic strains in isolates from nontoxigenic ones (1,13,14).

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In this study, we aimed to apply ELISA and LA tests in stool samples to determine the implication of *C. difficile* in the cases with diarrhea and the carriage rate of the bacterium in asymptomatic individuals.

MATERIALS AND METHODS

In this study, the stool specimens were collected between June 1996 and October 1997 from patients with or without diarrhea. The specimens were examined in the central laboratory of our hospital with serologic tests (LA, ELISA) and selective culture methods. A commercial LA kit (Becton Dickinson, USA) and CD-TOX *C. difficile* toxin A ELISA kit (Porton, Cambridge, England) were used to investigate presence or absence of *C. difficile* common antigen and toxin A in stool specimens following the manufacturer's recommended protocol and the ELISA plates were scanned using Bio-Tek EL-309 (Bio-Tech Instruments, USA). Fresh stool specimens from each patient are examined under the light microscope for fecal leukocytes. Stool samples also cultured on selective media (CCFA; cycloserine-cefoxitine egg-yolk fructose agar) and by the method of alcohol spore selection procedures. Selective culture plates were incubated at 37ºC anaerobically for 48-72 hours. Isolates of *C. difficile* were identified by API 20A (BioMeri France) system.

Age distribution of the patients is shown in Table 1.

Patients with diarrhea received the following antibiotics prior to the sampling date; clindamycin, ampicillin, cephalosporins, amoxicillin, and chloramphenicol. No patient had pseudomembranous colitis clinically. All patient specimens were also routinely cultured to identify other enteric pathogen bacteria (*Salmonella, Shigella, Campylobacter, etc.*). These cultures were positive in 16% of cases with diarrhea. *C. diffici* was isolated in four cases (4.1%) from 96 clinical samples from patients with diarrhea, and was isolated in three cases (3.4%) from 88 control samples. The results of *C. difficile* cultures are presented in Table 2.

Stool samples were examined under the microscope for fecal leukocytes. Fecal leukocytes were detected in 50% of cases with diarrhea. *C. difficile* was positive in 12 cases with ELISA and 17 with LA in the 184 stool samples examined. The positivity rates obtained in ELISA and LA tests were not found to be significantly (χ^2=2.176, p>0.05). The results are shown in Table 3.

When ELISA toxin A test together with bacterial cultures of toxigenic strains were accepted as standard test, the sensitivity of LA test was determined as 100%, its specificity as 97%, the predictive value of a positiv test (PPT) as 60%, and the predictive value of a negative test (PNT) as 100% (Table 4).

Table 1. Age distribution of age of the patients

	0-5		6-15		>16		Total	
	Number	%	Number	%	Number	%	Number	%
Cases with diarrhea								
Clinics	7	7.2	12	12.5	77	80.2	96	52.1
Policlinics	16	18.1	23	26.1	49	55.6	88	47.9
Total	23	12.5	35	19.1	126	68.4	184	100.0
Control cases	9	10.2	17	19.3	62	70.5	88	100.0

Table 2. The results of *C. difficile* positive cultures

	0-5		6-15		>16		Total	
	Number	%	Number	%	Number	%	Number	%
Cases with diarrhea								
Clinics	0	0.0	0	0.0	4	5.2	4	4.1
Policlinics	0	0.0	0	0.0	0	0.0	0	0.0
Control cases	0	0.0	1	5.8	2	3.2	3	3.4

Table 3. Positivity rates of *C. difficile* with ELISA and LA tests in stool samples

Method	Patient			Control		
	Number	+	%	Number	+	%
ELISA	184	12	6.5	88	0	0
LA	184	17	9.2	88	3	3.4

χ^2=2.176, p>0.05

Table 4. Comparison of ELISA and LA tests in stool samples

ELISA	LA		Total
	+	—	
+	12	0	12
—	8	252	260
Total	20	252	272

Predictive value of a positive test (PPT): 6o%
Predictive value of a negative test (PNT): 100%
Sensitivity: 100%
Specificity: 97%

DISCUSSION

The intensive use of antibiotics today for the control and treatment of infectious diseases have some unpredictable side effects. Antibiotics administered reduce the microbial diversity in gastro intestinal tract and may lead to diarrhea, and may also lead to complications by otherwise asymptomatically colonized bacteria (e.g., pseudomembranous colitis by *C. difficil*). The increasing incidence of *C. difficil* in the late 1970s put this microorganism into the group of important enteric pathogens. Although the spores of *C. difficil* are ubiquitous, they may colonize in the colon to the change of intestinal flora especially after the use of antibiotics. Althoug it is reported that all kinds of antibiotics may be associated with *C. difficil* colitis, it is believed that ampicillin, clindamycin, cephalosporins, aminoglycosides, and their combinations are among the main causes of the condition (7, 15, 16). Consistent with these reports, we observed that patients with diarrhea had used clindamycin, ampicillin, cephalosporins, amoxicillin, and chloramphenicol. However, factors apart from the use of antibiotics, such as geographical and socio-economic factors, age, gender and nutrition disorders may also cause *C. difficil* colonization and diarrhea in the gastrointestinal system (18).

There are toxigenic and nontoxigenic strains of *C. difficile.* Toxigenic strains produce two kinds of toxins with soluble protein structure sensitive to heat. These are A and B exotoxins (14). In the toxigenic strains of *C. difficile* another toxin with antigenically and physiologically different properties was identified and defin toxin C apart from toxin A and toxin B, but the contribution of this toxin to the pathogenesis has not been clearly shown (14).Twenty-five percent of human isolat do not produce toxins. Therefore, it was reported that the isolation only is not sufficient for physicians to s that *C. difficil* is responsible for the disease and the determination of its toxigenicity is mandatory (17). On the other hand, although the bacteria carried at a high rate in newborns produce toxins, they often do not cause any disease. The reason for this is that the intestinal cells are not probably sensitive to the effect of toxin in newborns and the sensitivity increases with age until the normal flora forms after the age of si -eight months. The prevalences of *C. difficil* carriage in healthy adults were reported to be 2% in Sweden and 15% in Japan. In the United States, nontoxigenic strains have been isolated in 2-33% in healthy asymptomatic adults. The rate of fecal carriage reaches 25-60% during the first months of life both in developed and developing countries however it decreases to 2-4% in healthy asymptomatic adults (17). However, the rate of fecal colonization has been shown to increase up to 21-56% in cases with diarrhea in developing countries especially as a result of using antibiotics (7, 16). The rate of *C. difficil* toxins isolation in feces among diarrhea patients with antibiotics history was reported at 11.9% in the United States, 3% in Sweden, and 14.5% in Australia. A study in Nigeria reported that although the colonization rate of *C. difficil* toxigenic strains was 15.6% in the stools of healthy adults, this rate was 6.7% in asymptomatic children (18).

Ovaran et al. (17) reported positive LA results in 19 (19.6%) cases among 97 stool samples of patients who diarrhea after antibiotic treatment in several clinics in Gata Haydar Paşa Hospital. In the same study, the toxin A was positive by ELISA in six cases (16.2%), LA in eight (21.6%), and culture was positive in five (among 37 cases. Di Persio et al. (12) investigated toxin B with culture, LA and cell culture, and toxin A with ELISA in 328 stool samples. In this study the positivity was observed in 52 cases (15.9%), using one or more tests. The 6.5% positivity rate in our study determined by toxin A investigation by ELISA in stool specimens from patients with diarrhea is in accordance with the findings of the similar studies conducted in our country and other countries.

In our study, ELISA and LA test were simultaneously performed. Higher positivity rate of LA depends on the sensitivity of this test being lower compared to ELISA. Furthermore, the nontoxigenic strains with this method and other anaerobic bacteria in stools are capable of giving cross-reaction. Although LA is not as sensitive as ELISA, it has been recommended as a screening test since it is less laborious, does not require special technical equipment, and is relatively rapid.

CONCLUSION

Based on the findings of our study, we suggest t *C. difficile* as well as other known etiologic agents should be carefully tested in the cases with diarrhea and that the tests for this microorganism should be included in the routine tests in clinical microbiology laboratories. Since immunologic procedures are fast and sensitive in the diagnosis of *C. difficil* , LA test for this microorganism should be primarily performed in clinical examples and ELISA toxin A test can be used to determine the toxigenity of the strains positive in LA test.

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