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Is the Incidence of *Clostridium difficile* in Nosocomial Diarrhoea Underestimated?

Nozokomiyal Diyarelerdeki *Clostridium difficile* İnsidansı Atlanıyor mu?

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SUMMARY

Introduction: *Clostridium difficile is a gram-positive, obligatory anaerobe, spore-forming microorganism, which is highly associated with nosocomial infections. In our hospital and our country, the incidence of nosocomial diarrhoea and C. difficile-associated nosocomial diarrhoea rates are not clear. Determining the C. difficile-associated nosocomial diarrhoea incidence, reviewing the current resistance status of C. difficile, and evaluating diagnostic and therapeutic approaches for this pathogen were the major aims of the present study.*

Materials and Methods: *This prospective clinical study included 100 diarrhoea samples from hospitalized patients in Istanbul University Cerrahpasa Faculty of Medicine. The diarrhoea samples were investigated by culture, card test and ELISA methods and bacterial resistance profiles were evaluated with the E-test method.*

Results: *Toxin A/B was found positive in 30/100 patients (30%) by ELISA. The duration of hospitalization and diarrhoea period were significantly longer in Toxin A/B positive patients than negative patients ($p< 0.05$). Recurrences were detected in 41% of toxin A/B positive patients (statistically not significant but clinically important). When ELISA was accepted as the main test, sensitivity and specificity of the culture and card test methods were found as 56%, 75% and 76%, 80%, respectively. C. difficile resistance rates were determined for metronidazole as 29.4% and for vancomycin and teikopilanin as 2.9%.*

Conclusion: *Our results support that C. difficile is still an important pathogen in nosocomial diarrhoea. Furthermore, the high rate of antibiotic resistance for metronidazole may cause difficulties in treatment. The results indicate the necessity of further studies to develop control measures and effective/reasonable treatment options for patients.*

Key Words: *Nosocomial diarrhoea; Clostridium difficile-associated diarrhoea; Antibiotic resistance*

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ÖZET

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Giriş: *Clostridium difficile'nin gram-pozitif, zorunlu anaerobik, spor oluşturan ve nozokomiyal infeksiyonlarla yüksek oranda ilişkili bir mikroorganizma odluğu bilinmektedir. Ancak, hastanemizde ve ülkemizde, nozokomiyal diyare ve C. difficile ile ilişkili nozokomiyal diyare oranları net olarak bilinmemektedir. Çalışmamızın temel amaçlarını; C. difficile ile ilişkili nozokomiyal diyare sıklığını belirlemek, C. difficile'nin mevcut direnç durumunu gözden geçirmek ve bu patojenle infekte hastalardaki tanı ve tedavi yaklaşımlarını değerlendirmek oluşturmaktadır.*

Materyal ve Metod: *Çalışmamıza, İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi hastanesinde yatan 100 hastadan alınan diyare örnekleri dahil edilmiştir. Diyare örnekleri kültür, kart test ve ELISA yöntemleriyle araştırılmış ve C. difficile'ye ait bakteriyel direnç profilleri E-test yöntemi ile değerlendirilmiştir*

Bulgular: *Çalışmaya dahil edilen 100 örnekten 30 tanesinde ELISA ile C. difficile Toksin A/B pozitifiliği saptanmıştır (%30). C. difficile toksin A/B pozitifliği saptanan hastalardaki yatış ve diyare sürelerinin ise toksin saptanmayan olgulara göre istatistiksel olarak daha uzun olduğu belirlenmiştir (p< 0.05). C. difficile toksin A/B pozitif olan hastaların %41'inde ise nüks infeksiyonu saptanmış, sonuçlar toksin A/B negatif hastalar ile kıyaslandığında istatistiksel anlamlılık saptanamasa da bu verinin klinik olarak önemli olabileceği düşünülmüştür. ELISA yöntemi temel test olarak kabul edildiğinde, kültür ve kart test yöntemlerinin C. difficile için hesaplanan duyarlılık ve özgüllük değerleri sırasıyla; %56, %75 ve %76, %80 olarak hesaplanmıştır. C. difficile direnç oranları metronidazol için %29.4, vankomisin ve teikopilanin için ise %2.9 olarak belirlenmiştir.*

Sonuç: *Sonuçlarımız, C. difficile'nin nozokomiyal diyarelerde hala önemli bir patojen olduğunu desteklemektedir. Ayrıca, metronidazol için saptanan yüksek direnç oranlarının, hastaların tedavisini zorlaştıran bir faktör olduğu düşünülmektedir. Sonuçlarımız, hastalar için kontrol önlemleri ve etkili/akılcı tedavi seçenekleri geliştirmeye yönelik daha ileri çalışmalara ihtiyaç duyulduğunu göstermektedir.*

Anahtar Kelimeler: *Nozokomiyal diyare; Clostridium difficile ile ilişkili diyare; Antibiyotik direnci*

INTRODUCTION

Clostridium difficile is the most common cause of healthcare-associated infectious diarrhoea. The spectrum of *C. difficile*-associated diseases ranges from diarrhoea to pseudomembranous colitis, and is frequently termed as *C. difficile*-associated diarrhoea (CDAD). All around the world, the incidence and severity of CDAD has increased, which appears to be caused by a number of factors such as large outbreaks of CDAD in hospitals, inappropriate antibiotic usage and performing inadequate hygiene techniques[1-4].

C. difficile is highly responsible for developing pseudomembranous colitis, antibiotic-associated colitis and antibiotic-associated diarrhoea with approximate rates of 90%, 75% and 33%, respectively[5]. In Turkey, the incidence rates of *C. difficile* in nosocomial infections are not clear. However, *C. difficile* has become an important pathogen in recent years due to failure in treatment detection in many hospitalized patients, increasing mortality rates, difficulties to control hospital outbreaks and changing antibiotic resistance profile of *C. difficile*. Despite sensitive diagnostic techniques, effective antibiotic treatments and healthcare infection control practices, *C. difficile* is still an important agent in nosocomial infections[6-8]. The aim of the present study was to determine the incidence of nosocomial diarrhoea in our hospital and to determine the role of *C. difficile*. Additionally, diagnostic techniques and antibiotic susceptibility for CDAD were investigated.

MATERIALS and METHODS

We examined stool samples from hospitalized patients sent to Istanbul University Cerrahpasa Faculty of Medicine laboratory over a 13-month period. The samples were firstly examined macroscopically to ensure that they were loose, watery, and the patients were questioned to confirm that they had a minimum three-days hospitalized period and that they were older than 18 years of age. One hundred samples meeting these criteria from 100 patients were included into our study. Permission to conduct this study was obtained from the local ethics committee of Istanbul University Cerrahpasa Medical Faculty Informed consents were obtained from all patients. Additionally, our study was performed according to the principles of the Helsinki Declaration.

Firstly, all samples were lightly inoculated on *C. difficile* selective agar (Oxoid, United Kingdom) and incubated at 37°C for 72 hours in Anaerobic Jar with an Anaerobic Gas Generating Kit (Oxoid, United Kingdom) to determine anaerobic and fastidious *C. difficile* colonies. After 72 hours, plates were evaluated in terms of the existence of *C. difficile* colonies, and *C. difficile* positive samples were transferred onto Iso-Sensitest Agar (Oxoid, United Kingdom) to determine the on-scale Minimum Inhibitory Concentration (MIC) of metronidazole, vancomycin, and teikoplanin with E-test strips (bioMurieux, France) by the recommendation of Clinical and Laboratory Standards Institute (CLSI). Enzyme-linked immunosorbent assay (Generic Assays, Germany) and immunochromatographic card test (Veda Lab, France) were used for the detection of *C. difficile* toxins A and B. All statistical analyses were performed using SPSS (Version 17.0 for windows) software by applying Student's t-test to determine the differences and Chi-square and Kappa values to determine the potential false-positivity and false-negativity.

RESULTS

One hundred patients were included into this study. Forty-eight of these patients were males and fifty-two were females. The average age and hospitalization time at the time of study of the 100 patients was 55 years (range 24 to 94 years) and 21 days (range 3 to 108 days), respectively. *C. difficile* toxin A or B was detected in 30 (30%) samples by ELISA method, hospitalization and diarrhoea period was significantly long in *C. difficile* toxin A or B positive group than the negative group ($p< 0.05$), and the results are shown in Table 1. Additionally, repetitive diarrhoea within 7 days after the inclusion of follow-up patients were evaluated as recurrence and the rates of recurrence were detected in 41% of *C. difficile* toxin A or B positive patients and were detected in 27% of *C. difficile* toxin A or B negative patients (statistically not significant but clinically might be important; $p= 0.165$). 74% of the included patients were noted positive for antibiotic usage. There was no correlation detected between the patients with previous antibiotic usage and *C. difficile* toxin A or B positivity ($p= 0.921$).

Conventional anaerobic culture, immunochromatographic card test and ELISA were used as diagnostic methods to determine the existence of *C. difficile* in diarrhoea samples. Because of its high sensitivity and specificity rates, ELISA was accepted as the reference test, and the sensitivity and specificity rates of the culture and card test methods were found as 56%-75% and 76%-80%, respectively (Table 2). Thirty-four *C. difficile* strains were grown in *Clostridium difficile* selective agar. *C. difficile* resistance rates were determined for metronidazole as 29.4% and for vancomycin and teikopilanin as 2.9%.

Table 1. Hospitalization and diarrhoea periods in toxin positive and negative groups

	ELISA negative		ELISA positive		
	Mean	Standard deviation	Mean	Standard deviation	p
Hospitalization duration (days)	16.93	21.456	31.70	30.663	0.021
Diarrhoea duration (days)	5.96	3.557	8.23	4.869	0.026

Table 2. Diagnostic values of culture and immunochromatographic card test when ELISA was accepted as reference test

	ELISA positive (n)	ELISA negative (n)	Total (n)	Sensitivity (%)	Specifity (%)	Kappa (κ) analysis
Culture positive	17	17	100	56%	75%	0.312
Culture negative	13	53				
Card test positive	23	14	100	76%	80%	0.531
Card test negative	7	56				

DISCUSSION

The incidence of *C. difficile* infections continues to rise and the infection is associated with increased morbidity and mortality in the elderly. In the United States, the incidence of *C. difficile* infection has doubled in the past 10 years[9]. Loo et al. have analyzed a dozen of hospitals in Canada and determined an incidence of 22.5 cases per 100.000 hospital admissions[10]. In the present study, the detected 30% positivity rate for *C. difficile* toxin A or B was found parallel with this findings, and also support that the incidence of CDAD continues to rise. The main causes of this increase might be connected with the rise in antibiotic resistance and lack of application of infection control measures.

The main risk factors associated to *C. difficile* are age older than 65, use of laxatives, proton pump inhibitors, chemotherapy, renal failure, gastrointestinal surgery, nasogastric tube, mechanical ventilation, prolonged hospital stay, and previous antibiotic therapy[11]. Predisposing factors to *C. difficile* infection include inappropriate antibiotic use, which is thought to alter the colonic flora, allowing *C. difficile* to proliferate. Many case reports suggest that previous antibiotic use is also related with *C. difficile*-associated diarrhoea[12-14]. In our study, there was no correlation detected between the patients with previous antibiotic usage and *C. difficile* toxin A or B positivity.

Different methods are used in the diagnosis of *C. difficile* infections, such as cell culture, stool culture, ELISA and card tests. Stool culture is not used due to its cost, to being labor intensive, and to the fact that the results take long to be obtained. Cell culture is the gold-standard method for the diagnosis of CDAD[15]. In the diagnosis of CDAD, enzyme immune assays are the most used laboratory methods, with results obtained in up to 2 hours. Nevertheless, depending on the exam methodology, sensitivity may vary between 50 and 99%, and specificity from 70 to 100%[16]. In the present study, card test and ELISA methods were used for the diagnosis of CDAD, and ELISA was preferred to detect toxin A or B positivity of *C. difficile* strains with its high sensitivity and specifity rates.

The rising incidence of CDAD since 2000 and the related extreme increases in severity, morbidity, and mortality have led to the improve of new agents to aid in disease prevention and treatment. These include new antibiotics for CDAD and also probiotic agents, bacteriotherapy, passive immunotherapy, and vaccine development[17]. In Israel, 49 patients with CDAD have been examined and metronidazole resistance rates have been found as 2%[18]. Moreover, Huang et al. have reported that many *C. difficile* isolates are still susceptible to vancomycin and metronidazole; however, transient and heteroresistance to MTZ and decreased sensivity have been determined. Resistance to antimicrobials in *C. difficile* varies widely between countries[19]. In our prospective study, *C. difficile* resistance rate to metronidazole was 29.4%, much higher than previously suggested in the literature. Our findings corroborate the alarming reports about the increasing metronidazole resistance rates of *C. difficile*.

In conclusion, *C. difficile* is one of the major complications related to healthcare and is easily spread at hospitals with its spore formation. The rising incidence and increased metronidazole resistance of *C. difficile* are alarming findings for hospitalized patients, especially in the elderly population. Patients with severe disease and/or patients treated in the intensive care units remain at high risk for this pathogen, and preventive measures, such as fastidious contact precautions, hand antisepsis, environmental disinfection, and, most importantly, antibiotic stewardship, are the cornerstones of the management *C. difficile*-associated infections.

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