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Is the incidence of clostridium difficile in nosocomial diarrhoea underestimated?

Özer Akgül[a]*, Burcu Sapmaz[b], Fadimana Çatal[c], Pelin Yüksel[d], Reyhan Çalışkan[d], Ömer Faruk Karasakal[a], Hayriye Kırkoyun Uysal[e]

[a]*Üsküdar University, Vocational School of Health Services, İstanbul, Turkey*
[b]*Nişantaşı University, Vocational School, İstanbul, Turkey*
[c]*Department of Medical Microbiology, T.C. Ministry of Health, Antalya Atatürk State Hospital, Antalya, Turkey*
[d]*Department of Medical Microbiology Istanbul University, Cerrahpaşa Medical Faculty, İstanbul, Turkey*
[e]*Department of Medical Microbiology, Istanbul University, Istanbul Medical Faculty, İstanbul, Turkey*

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*** Corresponding Author:**
Dr. Özer Akgül
Üsküdar University Vocational School of Health Services, Üsküdar/Istanbul, Turkey
e-mail: akgulozer@hotmail.com

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ABSTRACT

Clostridium difficile (C. difficile) is a Gram-positive, obligatory anaerobe, spore-forming microorganism and is highly associated with the nosocomial infections. The incidince of nosocomial diarrhoea and C. difficile-associated nosocomial diarrhoea rates are not clear in our country. To determine the C. difficile-associated nosocomial diarrhoea incidence, to review the current resistance status of C. difficile, and to evaluate diagnostic and therapeutic approaches for this pathogen were the aims of the present study. This prospective clinical study included 100 diarrhoea samples from hospitalized patients in İstanbul University Cerrahpaşa Medical Faculty of. The diarrhoea samples were investigated by culture, card test and ELISA methods and bacterial resistance profiles were shown with the E-test method. Toxin A/B was found positive at 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 detected in 41% of Toxin A/B positive patients (statistically not significant but clinically may be important). When ELISA was accepted as the main test, the sensitivity and specificity of culture and card test methods were found as 56%, 75% and 76%, 80%, respectively. The C. difficile resistance rates were determined for metronidazole as 29.4%, for vancomycin and teikopilanin as 2.9%. Our results support that the C. difficile is still an important factor in nosocomial diarrhoea. Furthermore, highness of antibiotic resistance for metronidazole may be caused by difficulties in treatment. The results indicate the necessity of further studies to develop control measures and effective treatment options for patients.

1.Introduction

Clostridium difficile (C. difficile) is the most common cause of healthcare associated infectious diarrhoea (Kelly and Lamont,2008). The spectrum of the Clostridium difficile-associated diseases ranges from diarrhoea to pseudomembranous colitis, and is frequently termed as C. difficile-associated diarrhoea (CDAD)(Khanna and Pardi, 2010). All around the world, the incidence and severity of CDAD has increased (Cartman et al., 2010). This increase 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 (Stuart and Marshall, 2011). C. difficile is highly responsible for developing pseudomembranous colitis, antibiotic-associated colitis and antibiotic-associated diarrhoea with approximate rates in 90%, 75% and 33%, respectively (Barbut et al., 2007). In Turkey, the incidence rates of C. difficile in nosocomial infections are not clear. However, C. difficile has become an important

pathogen in last years, because of the treatment failure detection in many hospitilazed patients, increasing mortality rates, diffuculties to control the hospital outbreaks and changing antibiotic resistance profile of C. difficile. Despite the sensitive diagnostic techniques, effective antibiotic treatments and healthcare infection control practices, C. difficile is still an important agent in nosocomial infections (Aygun et al., 2005; Cohen et al., 2010). 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.

2. Materials and Methods

Study Design

We prospectively examined stool samples from hospitalized patients over a 13-months period. The samples were firstly examined macroscopically to ensure that they were loose, watery, and the patients were questioned to confirm that had a minimum three-days hospitalized period and also older than 18 years old. One-hundred samples meeting these criteria from 100 patients were included in our study.

Methods

Firstly, all samples were lightly inoculated on Clostridium difficile selective agar (Oxoid, United Kingdom) and incubated at 37^{O}C for 72 hours in Anaerobic Jar with an Anaerobic Gas Generating Kit (Oxoid, United Kingdom) to determine the anaerobic and fastidious C. difficile colonies. After 72 hours, plates were evaluated in terms of the existence C. difficile colonies, and C. difficile positive samples were transferring on Iso-Sensitest Agar (Oxoid, United Kingdom) to determine the on-scale Minimum Inhibitory Concentration (MIC) of metronidazole, vancomycin, and teikoplanin with the E-test strips (bioMérieux, 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 detection of C. difficile toxins A and B.

Statistical Analyses

All statistical analyses were performed by using SPSS (Version 17.0 for windows) software by applying Student's t-test to determine the differences, Chi-square and Kappa values to determine the potential false-positivity and false-negativity. A p value of <0.05 was accepted as statistically significant.

Ethics

Permission to conduct this study was obtained from the local ethics committee of Istanbul University Cerrahpaşa Medical Faculty. Informed consents were obtained from all patients. Additionally, our study was performed according to principles of Helsinki Declaration.

3. Results

One-hundred patients were included in this study. Forty-eight of these patients were men and 52 were women. 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. There was no significant correlation in terms of genders and years of included patients C. difficile toxin A or B was detected in 30 (30%) samples by ELISA method, and the hospitalization time was significantly long in C. difficile toxin A or B positive group than the negative group ($p<0.05$). Addition, recurrences were detected in 41% of C. difficile toxin A or B positive patients ($p>0.05$, this is statistically not significant but clinically might be important).Conventional anaerobic culture, immunochromatographic card test and ELISA were used as diagnostic methods to determine the existence of C. difficile in diarrhoea samples. When ELISA accepted as the gold-standard test, sensitivity and specificity rates of culture and card test methods were found as 56%-75% and 76%-80%, respectively. Thirty-four C. difficile strains were grown in Clostridium difficile selective agar. The C. difficile resistance rates were determined for metronidazole as 29.4%, for vancomycin and teikopilanin as 2.9%.

4. Discussion

The incidence of C. difficile infections continues to rise and 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 (Tschudin-Sutter et al., 2012). Loo et al. analyzed a dozen of hospitals in Canada, and determined an incidence of 22.5 cases per 100,000 hospital admissions (Loo et al., 2005). In the present study, detected 30% positivity rate for C. difficile toxin A or B was found parallel with these findings, and also support that the incidence of CDAD continues to rise. The main causes of this rising might be connected with increase antibiotic resistance and lack of applying the 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 (Blondeau,2009). 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 would suggest that previous antibiotic use is also related with C. difficile-associated diarrhoea (Lundeen et al., 2007; Lavallée et al., 2009; Dineen et al., 2013). In our study, there were no correlation detected between the patients with previous antibiotic usage and C. difficile toxin A or B positivity.

Different methods are used to 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 diagnosis of CDAD (Musher and Aslam, 2008). In the diagnosis of CDAD, enzyme immune assays are the most used laboratory methods, with results in up to 2 hours. Nevertheless, depending on the exam methodology, sensitivity may vary between 50 and 99%, and specificity from 70 to 100% (Peterson et al., 2007). In the present study, card test and ELISA methods were used for the diagnosis of CDAD, and ELISA was preferred to detection the 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 caused 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 (Higa and Kelly, 2013). In Israel, 49 patients with CDAD examined and metronidazole resistance rates found as 2% (Bishara et al., 2006). Moreover, Huang et al. 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 (Huang et al., 2009). 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 metro nidazole resistance of C. difficile are alarming findings for hospitalized patients, especially in the elderly populations. Patients with severe disease and/or 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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