ORIGINAL REPORT

The Incidence of Nosocomial Toxigenic *Clostridium difficile* Associated Diarrhea in Tehran Tertiary Medical Centers

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Abstract- *Clostridium difficile* is the most common cause of nosocomial diarrhea. It is usually a consequence of antibiotic treatment, But sporadic cases can occur. This study was aimed to determine the frequency of the nosocomial *Clostridium difficile* (*C. difficile*) associated diarrhea in Tehran University of Medical Sciences hospitals and study of antibacterial susceptibility of isolates. In this study a total of 942 stool samples from patients with nosocomial diarrhea that were hospitalized in Imam Khomeini hospital, Shariati hospital and Children clinical center were collected. The samples were cultured on a selective cycloserine cefoxitin fructose agar (CCFA) and incubated in anaerobic conditions, at 37°C for 5 days. Isolates were characterized to species level by conventional biochemical tests. Bacterial cytotoxicity was assayed on tissue culture (vero). Antimicrobial sensitivity of isolated toxigenic *C. difficile* were investigated by kirby Beuer method (disk diffusion). Our findings show that, of the total patients, 57 toxigenic *C. difficile* (6.1%) were isolated. Results of statistical analysis show significant differences between the rate of isolated toxigenic *C. difficile* and age group of patients ($P<0.05$). Among the wards of selected hospitals, in gastroenterology of Children clinical center, Toxigenic *C. difficile* was isolated from patients most frequently. The sensitivity of isolates to vancomycin, Chloramphenicol and ceftriaxone were higher than other antibiotics. Toxigenic *C. difficile* is a common hospital-acquired infection. The organism was found in 6.1% hospitalized patients. Further studies to evaluate the rate and role of toxigenic *C. difficile* in nosocomial diarrheal processes, ecological and pathogenic terms are suggested.

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Key words: Nosocomial infection; diarrhea; Clostridium difficik; toxins, biological; antimicrobial sensitivity

Introduction

Clostridium difficile (C. difficile) is an anaerobic, gram-positive spore forming bacillus first isolated in 1935 from faecal flora of healthy neonates. This bactrium produces cytotoxin (toxin B) and enterotoxin (toxin A). It was not until 1978 that its association with antibiotic induced pseudomembranous colitis (PMC) was established (1). Isolation rate of C. difficile varies from 90% in PMC to 20-25% in AAD. Major risk factors include advanced age, duration of hospitalization, severity of underlying disease and exposure to antibiotics. The antibiotics most frequently associated are clindamycin, cephalosporin, ampicillin and amoxicillin. In a multivariate analysis, after making adjustments for other risk factors, these agents were found to be associated with highest risk of *C. difficile* diarrhea (2). Almost all antibiotics have been linked with C. difficile diarrhea and colitis, including vancomycin and metronidazole (which are used for its treatment) and cancer chemotherapy (3-4). The frequency of association is related to frequency of use, the route of administration and the impact of that antibiotic on the colonic microflora. This anaerobic bacterium has been identified as the leading cause of nosocomial infectious diarrhea and can be responsible for large out breaks. Nosocomial *C. difficile*- associated diarrhea include antimicrobial therapy, older age, antineoplastic chemotherapy and length of hospital stay. Other reported risk factors include presence of nasogastric tube and gastrointestinal procedures (2). The main goal of This study was to determine the Frequency of the nosocomial *C. difficile* -associated Diarrhea in Tehran University of Medical Sciences

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hospitals wards and study of antibacterial susceptibility of isolates .

Patients and Methods

In this study from December 2002 to February 2006, 942 stool specimens of patients with nosocomial antibiotic (erythromycin, tetracycline, gentamicin, ampicillin, amoxicillin, ciprofloxacine metronidazole,…) associated diarrhea that were hospitalized in three hospitals of Tehran University of Medical Sciences (Imam Khomeini hospital, Shariati hospital and Children clinical center) were collected. Selection criteria were antimicrobial therapy, long stay hospitalization, loose liquid stool and lack other enteric pathogens. Diarrhea was defined as three or more loose stools per day for at least 2 consecutive days. For isolation of *C. difficile* selective cycloserine cefoxitin fructose agar (CCFA medium, Bio Merieux, France) was used. Plated were incubated under anaerobic condition (N^2 = 80%; Co^2 10%; H^2 = 10%), for 48h at 37°C. The isolates were identified as *C. difficile* by characteristic morphology, horse odour, green-yellow fluorescence under UV light and biochemical test (API20A; Bio Merieux, France) including lipase, lecithinase, catalase, H_2S_2 and indole production; gelatin, esculin and starch hydrolysis; and glucose, fructose, lactose, maltose and sucrose, fermentation. Sensitivity of isolates to antibiotics were investigated by kirby Beuer method (disk-diffusion; Manufactured: Himedia, India) Bacterial cytotoxicity was assayed on vero (African green monkey kidney) tissue culture monolayers. A filter-sterilized, 1:10 dilution of feces was used to inoculate Vero cell monolayers with and without neutralizing *C. difficile* antitoxin (Tech Lab). Tissue cultures were examined at 24 and at 48h. Characteristic cytopathic effect (CPE) neutralized by antitoxin was interpreted as a positive result. Where a cytopathic effect was observed with a 1:10 dilution of feces and was not neutralized by antitoxin, the assay was repeated using a higher dilutions (1:40 and 1:100) of feces (5-11).

Results

Of total patients (525 males and 417 females, aged 1 months - 65 years), 57 toxigenic *C. difficile* (6.1%) were isolated (Table 1). The highest and lowest rate of isolated Toxigenic *C. difficile* was among age group of 1-10 years old (2.4%) and 31-40 years old (0.3%), respectively. Results of statistical analysis using Chi square test show significant differences between the rate of isolated toxigenic *C. difficile* and age group of patients (P<0.5) (Table 2).

Table 1. Frequency of patients with nosocomial diarrhea on the basis of sex and isolated toxigenic *C.difficile*

Toxigenic *C. difficile* / Sex	Positive	Negative	Total
Male	30 (5.7)	495 (94.2)	525 (55.7)
Female	27 (6.5)	390 (93.5)	417 (44.3)
Total	57 (6.1)	885 (93.9)	942 (100)

Table 2. Frequency of patients with nosocomial toxigenic *C. difficile* -associated Diarrhea on the basis of sex and age

Sex / Age (years)	Male	Female	Total
<1	6 (1.1)	9 (5.2)	15 (1.6)
1-10	14 (2.7)	9 (5.2)	23 (2.4)
11-20	3 (0.6)	2 (0.5)	5 (0.5)
21-30	2 (0.4)	3 (0.6)	5 (0.5)
31-40	2 (0.4)	1 (0.2)	3 (0.3)
>40	3 (0.6)	3 (0.6)	6 (0.6)
Total	30 (5.7)	27 (6.5)	57 (6.1)

Table 3. Antimicrobial susceptibility of isolated Toxigenic *C. difficile* from specimens of patients with nosocomial diarrhea

Antibiotics		Sensitive	Intermediate	Resistant
Chloramphenicol	(30 mcg)	75 (100)	0 (0)	0 (0)
Cefoperazone	(75 mcg)	47 (82.5)	8 (14)	2 (3.5)
Clindamycin	(2 mcg)	8 (14)	14 (24.5)	35 (61.4)
Vancomycin	(30 mcg)	57 (100)	0 (0)	0 (0)
Metronidazole	(30 mcg)	52 (91.2)	5 (8.8)	0 (0)
Ceftriaxone	(30 mcg)	57 (100)	0 (0)	0 (0)
Ciprofloxacine	(5 mcg)	21 (36.8)	21 (36.8)	15 (26.3)
Cefepime	(30 mcg)	46 (80.7)	5 (8.7)	6 (10.5)
Tetracycline	(30 mcg)	40 (70.1)	9 (14)	8 (14)
Kanamycin	(30 mcg)	0 (0)	0 (0)	57 (100)
Colistin	(10 mcg)	0 (0)	0 (0)	57 (100)
Gentamicin	(10 mcg)	0 (0)	0 (0)	57 (100)

Table 4. Frequency of patients with nosocomial diarrhea in Tehran tertiary medical center

Toxigenic *C. difficile* / Hospitals	Total		Negative		Positive	
	%	No	%	No	%	No
Imam Khomeini	24.9	235	94.9	223	5.1	12
Shariati	14.9	140	97.1	136	2.9	4
Children clinical center	60.2	567	92.8	526	7.2	41
Total	100	942	93.9	885	6.1	57

Table 5. Rate of isolated toxigenic *C. difficile* from 235 specimens of patients with nosocomial diarrhea in Imam Khomeini hospital wards

Toxigenic *C. difficile*	Wards							Total
	Internal medicine	Clinic	Gastroenterology	Dermatology	ICU	Infectious disease	Others	
Positive	3 (1.3)	3 (1.3)	2 (0.8)	1 (0.4)	1 (0.4)	2 (0.8)	_	12 (5.1)
Negative	44 (18.7)	43 (18.3)	38 (16.1)	5 (2.1)	1 (0.4)	18 (7.6)	74(31.5)	223 (94.9)
Total	47 (20)	46 (19.5)	40 (17)	6 (2.5)	2 (0.8)	20 (8.5)	74(31.5)	235 (100)

The sensitivity of isolates to chloramphenicol was 100%, cefoperazone 82.5%, Clindamycin 14%, Vancomycin 100%, Metronidazole 91.2%, Ceftriaxone 100%, Ciprofloxacine 36.8%, Cefepime 80.7%, Tetracycline 70.1%, Kanamycin 0%, Colistin 0% and Gentamicin 0%. The sensitivity of toxigenic *C. difficile* to chloramphenicol, Vancomycin and ceftriaxone were higher than other antibiotics (100%). The rate of intermediate resistance to metronidazole at the critical breakpoint (30 mcg) was 8.8% (Table 3). The rate of isolated toxigenic *C. difficile* among patients with nosocomal diarrhae in Imam Khomeini hospital, Shariati hospitals and Children clinical center were 12(5.1%, 5 male and 7 female); 4(2.9%,1 male and 3 female) and 41(7.2%, 24 male and 17 female), respectively. Among the wards of these three hospitals in gastroenterology of Children clinical center, toxigenic *C difficile* was isolated most frequently(2.8%) (Table 4-7).

Table 6. Rate of isolated toxigenic *C. difficile* from 140 specimens of patients with nosocomial diarrhea in Shariati hospital wards

Toxigenic *C. difficile*	Wards				Total
	Transplantation	Pulmonary	Gynaecology	Others	
Positive	2 (1.4)	1 (0.7)	1 (0.7)	-	4 (2.9)
Negative	43 (30.7)	6 (4.3)	24 (17.1)	63 (45)	136 (97.1)
Total	45 (32.1)	7 (0.5)	25 (17.8)	63 (45)	140 (100)

Table 7. Rate of isolated toxigenic *C. difficile* from 567 specimens of patients with nosocomial diarrhea in Children clinical center wards

Toxigenic C. difficile	Wards						Total
	Infectious disease	Surgical	Gastroenterology	Nurology	Haematology and Kidny	Clinic	
Positive	6 (1.1)	7 (1.2)	16 (2.8)	3 (0.5)	5 (0.9)	4 (0.7)	41 (7.2)
Negative	106 (18.7)	54 (9.5)	243 (42.8)	43 (7.6)	2 (0.3)	78 (13.8)	526 (92.8)
Total	112 (19.8)	61 (10.7)	259 (45)	46 (8.1)	7 (1.2)	82 (14.5)	567 (100)

Discussion

During the past 20 years, *C. difficile* has emerged as a major cause of antibiotic associated diarrhea, nosocomial diarrhea and has been responsible for large out breaks in hospital settings (12-13). *C. difficile*: toxin B, has been isolated from stools of more than 95% of PMC cases and of 15-25% cases of antibiotic-associated diarrhra (14).

Different studies showed that *C. difficile* was a major agent of nosocomial diarrhea in adults (15-19). In 260 adults under the care of their general practitioner showed that antibiotic-associated diarrhea is frequent and is observed in 17.5% of patients. Among these patients, a toxigenic strain of *C. difficile* has been found in 8.7% of cases. The incidence of *C. difficile*–associated diarrhea among hospitalised patients has been found to vary widely, from 0.1 to 2% (20). These incidences include patients with severe *C. difficile*-associated diarrhea who required hospitalisation and patients with *C. difficile* nosocomial infection. In the study performed by olson et al. (21), the total number of *C. difficile* cases in 10 years was 908, leading to an annual incidence ranging from 0.4 to 1%. In this study, 93% of cases were classified as nosocomially acquired. Surveillance data from one Turkish hospital indicated that nosocomial diarrhea was ranked fifth and comprised about 7% of all nosocomial infections according to centers for disease control 1988 criteria (22). Furthermore, in this hospital, *C. difficile* was responsible for 22.2% of the nosocomial diarrhea cases that occurred within a general medicine ward (23).

Outbreaks of *C. difficile* – associated diarrhea have occurred on geriatric wards (24). Orthopedic wards (25), medical wards (26), surgical wards (27) and long-term care facilities (28). The incidence rate of nosocomial *C. difficile* – associated diarrhea may vary with hospital populations and is influenced by the presence of predisposing factors, such as increased patient age, type and duration of antimicrobial therapy, severity of underlying illnesses and length of hospital stay. Nosocomial infection with *C. difficile* increases morbidity and mortality among hospitalized patients and places a significant economic burden on healthy services (28-33). As the most frequently isolated nosocomial gastrointestinal pathogen, *C. difficile* is believed to be the leading cause of infectious nosocomial diarrhea, accounting for 20 to 45% of all cases (34-35). Despite some geographical variation, diarrhea is considered to account for 1 to 14% of all nosocomial infections throughout the world (36).

In our study 57 patients (6.1%), hospitalised in Tehran University of Medical Sciences hospitals with nosocomial antibiotic(erythromycin, tetracycline, gentamicin, ampicillin, amoxicillin, ciprofloxacine metronidazole,…) associated diarrhea were positive and 885 negative for Toxigenic *C. difficile*. The number of specimens of patients with nosocomial diarrhea with and without toxigenic C. difficile in Imam Khomeini hospital, Shariati hospital and Children medical center were 12and 223, 4 and 136, 41 and 526, respectively. These bacteria were mainly isolated from patients in gastroenterology of Children clinical center (2.8%). Our results confirmed presence of Toxigenic *C. difficile* as important etiological agent of nosocomial diarrhae in Tehran University of Medical Sciences hospitals. Results of antimicrobial sensitivity showed that, all isolated *C. difficile* were susceptible to chloramphenicol, vancomycin and ceftriaxone. The rate of intermediate resistance to metronidazole was 8.8%.

Use of rapid and sensitive techniques for laboratory diagnosis, a change in antibiotic policy, tight restriction of unnecessary antibiotic use, especially broad-spectrum ones and implementation of standard infection control measures is necessary to reduce morbidity due to *C. difficile* associated infections in hospitalised patients.

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