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Toxigenic *Clostridium difficile* Infection in Children: Performance of Toxin A/B Immunoassay

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Abstract

Objectives: *Clostridium difficile* is a major nosocomial pathogen that infects the large intestine (colon) and causes a range of clinical manifestations from mild to severe diarrhea. The aim of this study was to assess the detection of pathogenic toxin A/B-positive *C. difficile* strains among hospitalized patients with diarrhoea in Gonabad, Iran.

Materials and Methods: In this cross-sectional study, hospitalized patients submitted to 22 Bahman Hospital (Gonabad, Iran) were included if they had a liquid stool specimen. From November 2016 through July 2017, a total of 50 sequential stool samples (unformed or liquid) were obtained from hospitalized patients for inclusion in this study. The stool samples were collected and *C. difficile* toxin A/B immunoassay was performed according to the manufacturer's guidelines.

Results: Based on the results, the maximum number of cases belonged to the age group of less than 10 years (n=29, 58%). Most studied patients were hospitalized in the pediatrics ward (n=24, 48%). Among the studied patients, 16 (32%) cases were positive for *C. difficile* toxin A/B by immunochromatographic assay. Frothy-four (88%) patients received one or more antibiotic treatment on admission and hospitalization. Ceftriaxone was the most common applied drug for the treatment of these patients. Finally, 5 (31.25%) patients received combination therapy. Overall, 34.48% of pediatric cases were positive for *C. difficile* toxin A/B.

Conclusions: Our results showed that the emergence of potentially highly virulent and antibiotic-resistant *C. difficile* isolates is alarming. The diagnostic of pathogenic *C. difficile* by the clinical microbiology laboratory can improve the handling of disease and more reasonable use of antimicrobials by the physician.

Keywords: Hospitalized patients, Children, Diarrhea, Toxigenic *Clostridium difficile*

Introduction

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacillus that infects the large intestine or colon (1). It is an important nosocomial bacterium causing a range of clinical manifestations from mild to severe diarrhea (2) and a wide spectrum of gastrointestinal complications such as toxic megacolon, pseudomembranous colitis, and diarrhea (1). The number of *C. difficile* infections (CDIs) have been rising during the last decade, and CDI is an important healthcare concern and a cost factor in community and hospital settings (3). Isolates with high pathogenesis potency and virulence determinants have been identified currently (4).

The pathogenicity of this bacterium is related to the production of two large bacterial toxins including toxin A (TcdA, 308 kDa) and toxin B (TcdB, 270 kDa). These toxins are identified as the major pathogenesis determinants of *C. difficile* and inflammation, fluid secretion, and colonic tissue damage associated with infection (5). Nontoxigenic strains are not pathogenic. TcdA is a bacterial enterotoxin whereas TcdB is a multi-functional bacterial cytotoxin (5,6). Both of these bacterial toxins can cause mucosal damage and act on their own or synergistically (6). TcdB toxin is a bacterial peptide with the main task in *C. difficile*-associated disease pathogenesis and TcdB induces rapid necrosis in host epithelial cells (7). Experiments with purified toxins have revealed that toxin A alone is able to evoke the clinical manifestations of CDI whereas toxin B is unable to perform this process unless it is mixed with toxin A or there is prior damage to the layer of the intestinal mucosa (8). In contrast to adult individuals in whom the rate of colonization in the community and hospital settings is 3% and 20%, respectively, the prevalence of *C. difficile* colonization in neonates ranges from 2% to 50% and colonization often occurs within the first week of life (9).

Clostridium difficile is one of the most common nosocomial bacteria in the industrial nations and the major etiological agent of antibiotic-associated diarrhea (10). Diarrhoea diseases are a relatively common side effect of antimicrobial treatments (11). The use of expended-spectrum drugs such as β-lactams (e.g., cephalosporins

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Key Messages

- One of the interesting findings in our study was that 3 C. difficile toxin A/B positive patients received no antibiotic, all these cases were in the age group of ≤ 10 years.

and penicillins), macrolides (e.g., erythromycin, azithromycin, and clarithromycin), fluoroquinolones, and clindamycin disrupts the normal intestinal flora, predisposing patients to colonization by *C. difficile*, which is mainly encountered in health care centers (12). It should be noted that the asymptomatic carriers of *C. difficile* (about 2% of healthy adults, 50% of neonates, and 20% of hospitalized patients) usually outnumber symptomatic patients. Thus, the presence of patients under antibiotic treatment coupled with a high number of healthy carriers among hospitalized patients explains the high rate of nosocomial diarrhea related to *C. difficile* (13, 14).

CDI is a remarkable agent of mortality and morbidity in the healthcare wards. The number of patients experiencing recurrent infections is rising in Iran (3, 15). The diagnosis of CDI needs the assessment of both clinical and laboratory findings. Rapid, commercially available, toxin A/B detection kits have eliminated the need for clinical laboratories to maintain the cell lines necessary for cytotoxicity assay. There are currently available kits for the detection of both TcdA and TcdB in isolates. A specific, rapid, simple diagnostic test would be useful to the physician for the management of illness and the use of an effectual therapy at the onset of the disease for the prevention of morbidity and mortality. In this regard, this study sought to assess the detection of pathogenic toxin A/B-positive *C. difficile* strains among hospitalized patients with diarrhoea in Gonabad, Iran. To the best of our knowledge, the present study was the first one on the toxigenic *C. difficile* infection in this geographical region.

Materials and Methods

Patients

Hospitalized patients submitted to 22 Bahman hospital (Gonabad, Iran) and if they had a liquid stool specimen were included in this cross-sectional study. Before sample collection, all study populations were registered with a code number. The demographic information of patients and prescribed antibiotics were recorded, and stool specimens were sent by the clinician to the clinical microbiology laboratory for *C. difficile* testing using the immunochromatographic method.

Sample Collection and Storage

A total of 50 sequential stool samples (unformed or liquid) from hospitalized patients were collected for inclusion in this study from November 2016 to July 2017. The stool samples were collected in clean containers without any additives and shipped to a microbiology laboratory in batches. The samples were stored at -20°C before beginning the test for *C. difficile* toxin A/B immunoassay by standard protocols according to manufacturer's guidelines.

Clostridium difficile Toxin A/B Immunoassay

The samples, test cassettes, and reagent vials were brought to room temperature (20-25°C) and completely thawed prior to beginning testing. RIDA®QUICK *C. difficile* toxin A/B immunoassay (R-Biopharm AG, An der Neuen Bergstraße 17, D-64297 Darmstadt, Germany) was performed on all samples according to the manufacturer's recommendations. The results were interpreted according to the manufacturer's guidelines. All *C. difficile* toxinA/B detection assays were done on the same day for all stool specimens.

Statistical Analysis

All data were statistically analyzed by SPSS software (version 19.0) and presented as percentages. Pearson's chi-square test was used to compare the frequency. *P* values of ≤0.05 were assumed to be statistically significant. Standard deviations and means were calculated as required for numerical variables.

Results

Patients' Data

In the current study, 50 patients with liquid stools sent for *C. difficile* toxin A/B assay were enrolled in the study. Table 1 presents the demographic data of the studied patients consisting of 18 males (36%) and 32 females (64%). Based on the obtained data (Table 1), the maximum number of cases belonged to the age group of less than 10 years (n = 29, 58%). Fifteen (30%) patients had higher than 40 years of age. Most studied patients were hospitalized in the pediatrics ward (n = 24, 48%). Gastroenteritis was the most common clinical manifestation among the patients. Forty-seven (94%) patients were from Gonabad (Iran). Among the studied patients, 16 (32%) cases were positive for *C. difficile* toxin A/B by immunochromatographic assay.

Table 2 provides different antibiotics used for the treatment of studied patients by the physician. The analysis of data revealed that 44 (88%) patients received one or more antibiotic treatment on admission and hospitalization (17 patients: ceftriaxone; 3 patients: metronidazole; 3 patients: ciprofloxacin; 3 patients: ceftriaxone + metronidazole). In addition, cefotaxime + ampicillin was administered to 2 patients.

Table 3 presents the characteristics of the A/B toxin positive patients including 1 male (6.25%) and 15 females (93.75%). A/B toxin positive patients' ages ranged from 5 months to 89 years, of which 1 case was 38 years (6.25%). Most patients were ≤10 years old. Nine (56.25%) patients were hospitalized in the pediatrics ward. Diarrhea was the most common (56.25%) clinical manifestation in A/B toxin positive cases. Ceftriaxone was the most

Table 1. Demographic Features of the Studied Patients (N=50)

Category	No. (%)
Gender	
Male	18 (36)
Female	32 (64)
City	
Gonabad	47 (94)
Other cities	3 (6)
Age (y)	
0-10	29 (58)
11-20	3 (6)
21-30	0 (0)
31-40	3 (6)
>40	15 (30)
Hospital wards	
Pediatrics	24 (48)
Emergency	12 (24)
General internal medicine	10 (20)
ENT	4 (8)
Clinical manifestations	
Diarrhea	14 (28)
Gastroenteritis	23 (46)
Diarrhea + fever	6 (12)
Diarrhea + fever + vomiting	2 (4)
Fever	3 (6)
Sepsis	2 (4)
Clostridium difficile toxin A/B assay	
Toxin A/B positive	16 (32)
Toxin A/B negative	34 (68)

Note. ENT: Ear, nose, throat ward.

common drug used for the treatment of these patients, seven patients (43.75%) received ceftriaxone as the drug of choice. Five (31.25%) patients received combination therapy (ceftriaxone + metronidazole, ceftriaxone + cotrimoxazole, and ceftriaxone + azithromycin). No drug was prescribed for 3 (18.75%) patients.

Table 4 provides the feature of pediatrics that were *C. difficile* toxin A/B positive. Overall, 34.48% of pediatric cases were *C. difficile* toxin A/B positive. Patients' ages ranged from 5 months to 10 years, of which only 1 case was male. All patients were living in Gonabad (Iran). One of the patients was hospitalized in the emergency ward. Two

Table 3. Demographic Characteristics of the *Clostridium difficile* Toxin A/B Positive Patients (n=16)

Category	No. (%)
Gender	
Male	1 (6.25)
Female	15 (93.75)
Age (y)	
≤10	10 (62.5)
31-40	1 (6.25)
81-90	5 (31.25)
Hospital ward	
Pediatrics	9 (56.25)
Internal medicine	4 (25)
Emergency	3 (18.75)
Clinical sign	
Gastroenteritis	5 (31.25)
Diarrhea	9 (56.25)
Diarrhea-fever	2 (12.5)
Prescribed drugs	
Ceftriaxone	7 (43.75)
Ceftriaxone + metronidazole	2 (12.5)
Ceftriaxone + cotrimoxazole	2 (12.5)
Ceftriaxone + azithromycin	1 (6.25)
Cefotaxime	1 (6.25)
Not prescribed	3 (18.75)

cases showed gastroenteritis. Among the studied patients, 3 cases received no drug during the hospitalization period.

Discussion

Considering that *C* infection has become increasingly more rampant in the community and has developed as a common hospital-acquired infection, the accede to diminish underlying agents, risk factors, and handling has become more incentive needing novel guidelines (15). It is well-identified that the toxinA/B assay is helpful for CDI diagnosis since toxin production by *C. difficile* is thought to be one critical factor associated with CDI progress and intensity.

In the present cross-sectional study, the prevalence of pathogenic *C. difficile* (toxin A/B positive) strains was assessed among hospitalized patients with diarrhoea in Gonabad (Iran). Most studied patients were hospitalized in the pediatrics ward (n = 24; 48%), who were followed by internal medicine (n = 12, 24%). Most cases were children (n = 29, 58%). In our study, 16 (32%) cases were

Table 2. Antibiotics Used for the Treatment of the Studied Patients (N=50)

Antibiotic	CRO	MZ	CIP	CRO + CIP	CRO + MZ	CRO+CD	CTX+AP	CRO + AZT + CIP	CRO+SXT	CRO+AZT	GM	CRO+VA + IMI	CTX	NP
Total (%)	17 (34)	3 (6)	3 (6)	2 (4)	3 (6)	2 (4)	2 (4)	2 (4)	2 (4)	2 (4)	1 (2)	1 (2)	2 (4)	6 (12)

Note. CRO: Ceftriaxone; MZ: Metronidazole; CIP: Ciprofloxacin; CD: Clindamycin; AP: Ampicillin; CTX: Cefotaxim; AZT: Azitromycin; SXT: Cotrimoxazole; GM: Gentamicin; VA: Vancomycin; IMI: Imipenem; NP: Not prescribed.

Table 4. Characteristics of the *Clostridium difficile* Toxin A/B Positive Pediatrics (n = 10)

Patient Code	Age	Gender	City	Hospital	Hospital Ward	Clinical Manifestation	Antibiotic
1	4 years	Female	Gonabad	22 Bahman	Pediatrics	Diarrhea	Not prescribed
8	2 years	Female	Gonabad	22 Bahman	Pediatrics	Gastroenteritis	Not prescribed
9	3 years	Female	Gonabad	22 Bahman	Pediatrics	Gastroenteritis	Not prescribed
14	5 months	Female	Gonabad	22 Bahman	Pediatrics	Diarrhea	Cefotaxime
27	8 years	Female	Gonabad	22 Bahman	Pediatrics	Diarrhea	Ceftriaxone
32	9 months	Female	Gonabad	22 Bahman	Pediatrics	Fever, Diarrhea	Ceftriaxone + cotrimoxazole
33	12 months	Male	Gonabad	22 Bahman	Pediatrics	Diarrhea	Ceftriaxone
36	10 years	Female	Gonabad	22 Bahman	Emergency	Diarrhea	Ceftriaxone
48	12 months	Female	Gonabad	22 Bahman	Pediatrics	Diarrhea	Ceftriaxone
49	5 years	Female	Gonabad	22 Bahman	Pediatrics	Fever, diarrhea	Ceftriaxone + metronidazole

positive for *C. difficile* toxin A/B among whom, 10 cases were children. Various studies introduced the increased incidence of CDI among children. Variations in CDI in children during the past decade included the emergence of *C. difficile* isolates, which are hypersporulate, increasing the numbers of cases with severe disease, and a rising incidence of community-onset *C. difficile* infections (16).

In our study, among *C. difficile* toxin A/B positive cases, 6 patients were in the age range of 1-10 year(s) old. More recently, prospective experiments have shown that the *C. difficile* colonization rate in children younger than 2 years is 33% (17). Numerous opinions have been suggested for clarifying the overall rise in CDI. These alternatives include inadequate prevention measures in healthcare settings and raised usage of broader spectrum antibacterial antibiotics (e.g., beta-lactams, fluoroquinolones, and macrolides), and hospitalized patients who are often older and sicker (18).

In one observational research on children, 25% of *C. difficile*-positive stool specimens showed asymptomatic colonization. Among healthy adult individuals in the human community, the intestinal carriage rates range from 1.6% to 4% for toxigenic *C. difficile* isolates in recent research. Colonization rates among hospitalized adult patients vary between 5% and 26% (17).

In the present study, data analysis demonstrated that 4 *C. difficile* toxin A/B positive cases are ≤12 months. The importance of *C. difficile* among the infant group remains controversial. The early peak of colonization in infants is over the first month of life. Previous evidence indicated the rates of asymptomatic colonization with *C. difficile* up to 70% among the healthy infant group (17). The neonate group is uniquely susceptible to *C. difficile* colonization because of the lack of protective intestinal microbiota and the immaturity of the neonatal intestine. The lack of *C. difficile* infection in neonates is associated with diminished or immature receptor sites for toxin A attachment to the cell host (17,19).

One of the interesting findings in our study was that 3 *C. difficile* toxin A/B positive patients received no antibiotic, all these cases were in the age group of ≤ 10 years. CDI is related to the consumption of antibiotics although the number of cases with diarrheal signs and those recognized as CDI without any prior antibiotic exposure is rising (15,20). Moreover, the community isolates of *C. difficile* are emerging (21). It is estimated that community-acquired CDI may now account for up to 40% of CDI patients (15,22).

Among the studied patients, 17 (34%) cases received ceftriaxone that among whom 7 (43.75%) patients were positive for *C. difficile* toxin A/B. Different antibiotics, especially from broad-spectrum antibiotic classes are related to CDI. However, the use of proton pump inhibitor drugs as treatment is rising among the outpatients because the long-term consumption of proton pump inhibitors may raise the risk of CDI (23). Based on our results, 2 (12.5%) *C. difficile* toxin A/B positive patients received metronidazole although the emergence of potentially highly virulent and antibiotic-resistant *C. difficile* isolates is alarming.

To the best of our knowledge, this is the first toxin assay study for the diagnosis of *C. difficile* infection by toxin detection directly from stools in Gonabad, Iran. The diagnostic of pathogenic *C. difficile* may lead to more reasonable use of antimicrobial drugs and complete the handling of the disease. In addition, the prompt identification of patients with CDI is imperative for infection prevention and control and antibiotic stewardship. Nonetheless, our findings forcefully support the usage of toxin assay testing as the primary diagnostic laboratory test for the detection of CDI.

It should also be noted that in many cases, microorganisms are transmitted to humans, hospital environments, and hospitalized patients through other sources including animals, food plants, poultries, fish, and other industries (24-26). However, we require to refocus our management and prevention protocols on community and hospital wards.

Conclusions

Future studies will focus on identifying novel diagnostic tests, more effective antibiotics, and new treatments such as vaccines, fecal transplantation, and monoclonal antibodies. This is of special importance in pediatric practice due to the known high levels of colonization with this bacterium in young children. Furthermore,

community-acquired *C. difficile* infection has emerged, thus we require to develop these strategies for the household. In conclusion, CDI diagnosis should be according to the background of the clinical manifestations of the patient in correlation with clinical laboratory results for the detection of *C. difficile*.

Authors' Contribution
All listed authors have equally contributed to the project.

Conflict of Interests
The authors declared that they have no competing interests.

Ethical Issues
The project has been approved by ethics committee of Gonabad University of Medical Sciences (grant No. 94/48).

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