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ORIGINAL ARTICLE

Frequency of *Clostridium difficile* among patients with gastrointestinal complaints

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ABSTRACT

Aim: In this study, the prevalence of *C. difficile*, from patients with gastrointestinal complaints and its association with other enteropathogen microbes were investigated.

Background: *Clostridium difficile* is an important pathogen associated with outbreaks of pseudomembranous colitis and other intestinal disorders, such as diarrhea.

Patients and methods: Enterotoxin and cytotoxin (toxin A and toxin B) of *C. difficile* on the patient's stool samples were detected by a double sandwich enzyme-linked Immunosorbant assay technique using a commercial kit (Premier toxins A & B; Generic Assays, Inc., Germany). The microbial isolation and examination was done, according to the standard identification methods.

Results: Out of 356 individuals (57.6 % male and 42.4 % female) the results of *C. difficile* were positive for 19 patients (5.3 %) and negative for 337 patients (94.6 %) according to the results of *C. difficile* antigen kit. There was no association between the existence of *C. difficile* toxin and microbial population or antibiotic usage.

Conclusion: This prevalence study clearly supports the hypothesis of a probable role of *C.difficile* in developing gastrointestinal complaints in patients with diarrhea. More studies are needed to evaluate the role of *C. difficile* in these diseases.

Keywords: *Clostridium difficile*, Gastrointestinal complaints, Iran.

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Introduction

Clostridium difficile (*C.difficile*) is responsible for more than 90% of cases of pseudomembranous colitis and approximately 25% of diarrhea cases observed during or after antibiotic therapy (1). Clinical manifestations of infection range from asymptomatic colonization, to severe diarrhea, pseudomembranous colitis (PMC), toxic megacolon and death (2).

C.difficile produces two toxins, toxin A (enterotoxin) and toxin B (cytotoxin). These are thought to be the primary causes of colonic mucosal injury and inflammation. Strains of *C.difficile* that do not produce the toxins are not pathogenic. The importance of detecting toxin B relative to toxin A has been extensively investigated. Initially, toxin A was reported to be responsible for the disease (3). However, the role of toxin B has been reviewed and toxin B is now considered more important than toxin A, and possibly essential for virulence (3-6). Rapid diagnosis of this pathogen is decisive in allowing

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clinicians to prescribe the appropriate therapy and to take adequate measurement for controlling nosocomial spread. Various laboratory methods may be used to detect the presence of *C. difficile* or its related toxins (7, 8). In Iran, only a small number of clinical laboratories are able to reach a definitive diagnosis of *C. difficile* infection, because simple reliable assays for toxins in isolates are not available. The gold standard for diagnosis is a tissue culture assay for the cytotoxicity of toxin B, utilizing pre-incubation with neutralizing antibody against this toxin to show the specificity of cytotoxicity. This test can detect as little as 10 picograms of toxin B in stool and has a high sensitivity (94-100%) and specificity (99%). However, the test takes 1-3 days to complete and requires tissue culture facilities. More recently, enzyme linked immunosorbent assays (ELISAs) have been developed to detect toxin A and/or B in stool. These assays detect 100-1000 picograms of either toxin and have a sensitivity of 66-94% and a specificity of 92-98% (7-11).

We belive a local prevalence study of an infectious disease in our community is an initial step towards the introduction of the proper interventions for controlling the disease. This report has invstigated on the frequency of *C. difficile* infection and any correlation between infection and gastrointestinal symptoms or disorders.

Patients and Methods

A total of 356 stool samples were collected from patients who had been referred to the department of foodborne and diarrheal diseases, Taleghani Hospital, Tehran. Patient complaints included abdominal pain, flatulence, tenesmus, diarrhea and dysentery. Stool samples were cultured for pathogenic bacteria and fungi. Presence of *cryptosporidium spp. and* other parasites were also investigated by modified acid-fast stains and other standard laboratory methods, respectively. Detection of enterotoxin and cytotoxin (toxin A and toxin B) of *C. difficile* was performed on the stool specimens by a double sandwich enzyme-linked Immunosorbant assay technique using a commercial kit (Premier toxins A & B; Generic Assays, Inc., Germany). The assay was performed according to the manufacturer's instructions.

Results

Out of the 356 individuals (57.6 % male and 42.4 % female) referred to the department of foodborne and diarrheal diseases, *C. difficile* were positive for 19 (5.3 %) patients. Table 1 indicated the distribution of *C.difficile* in patients with gastrointestinal complaints according to gender.

Table 1. Gender distribution of *C. difficile* in patients with gastrointestinal complaints

	positive		negative		total	
	no	%	no	%	no	%
Male	13	6.3	192	63.6	205	57.6
Female	6	4	145	96	151	42.3
total	19	5.3	337	94.6	356	100

No enteric pathogen including bacteria and intestinal parasite were founded. The most common non-pathogen parasite was E.coli (5.1%) followed by I.bottcheli (2.2%).

Table 2. Distribution of gastrointestinal symptoms in patients infected by *C. difficile* comparet to non-infected

Symptoms	**positive**	**negative**
abdominal pain	20%	45%
Diarrhea	45 %	32%
Nausea	5.2%	14.6%
Vomiting	8.2%	4.2%
other related symptoms	38.5%	28.6%

All 356 subjects present GI symptoms, including abdominal pain (60%), diarrhea (31.5 %), nausea (28.5 %), vomiting (10.2 %) and other related symptoms (48.5%). Of 356 patients recruited stool samples, 284 were reported as normal stool and 72 (20.22%) as diarrheic stool.

Of 356 patients, 23 (4.6%) patients were taken antibiotics during the study. Distribution of gastrointestinal symptoms in patients infected by *C. difficile* compare to non-infected.

Discussion

C. difficile is an organism that can be found in most of people's without causing symptoms, but in some people it can cause a severe colitis. Predisposing actors include antibiotic therapy. the *C difficlie* toxin damages the fragile lining of the bowel causing loose watery bowel movements (diarrhea) (12). The organism is usually acquired from the hospital, as environmental contamination is common and health care workers may carry it in their hands, or on contaminated instruments.

Due to the rapidity of testing and ease of performance, ELISAs for toxin A and B are now used most frequently by clinical laboratories for diagnosis of *C. difficile* infection. In our study *C. difficile* infection was detected in 5.3 % of patients with gastrointestinal complaints. According to a previous study in Iran, *C. difficile* was isolated from 6.1% of patients with nosocomial diarrhea and 4% in children with diarrhea in Iran (13, 14). This amount was reported 4.9% for Turkish patients with diarrhea (15). Only 4.6% of the patients with toxigenic *C. difficile* were taking antibiotics at the time of the sampling and 96.4% of them have not used antibiotics. These results contrast with the another study in Iran, that reported that all of their hospitalized patients with toxigenic *C.difficile* positive stool samples have taken antibiotics at the time of the sampling (13).

In Brazil, *C. difficile* was detected in 5.5% of hospitalized children with acute diarrhea and in Argentina 38.5% *C. difficile* positive samples were detected from symptomatic patients (4, 16).

Our study has a number of limitations. We used an ELISA test for screening that has a high specificity (99–100%) but a low sensitivity (75–85%) to detect *C.difficile* toxins A and B (5, 11). Our study showed that *C.difficile* was potentially the pathogen responsible for gastrointestinal complaints in a significant proportion of patients who denied antibiotics therapy, prior to their admission to hospitals.

As a consequence it seems necessary to investigate the mechanisms involved in the infection and pathogenesis of this organism within different populations and different host microenvironments.

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