# Frequency of *Clostridium Difficile* Infection in Hospitalized Children in Suez Canal University Hospital

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## Abstract

***Background: Clostridium difficile (C. difficile),* a Gram-positive spore-forming bacillus, is the most common etiologic agent of antibiotic-associated diarrhea. The clinical presentation of *Clostridium difficile*-associated disease (CDAD) occurs secondary to the production of two exotoxins; toxin A, an enterotoxin and toxin B, a cytotoxin. *Aim:* to assess the frequency of *C. difficile* infection in hospitalized children in Suez Canal University Hospital for the purpose of prevention and control. *Patients and Methods:* This study was conducted as a cross-sectional study to detect *C. difficile* infection in 90 hospitalized children in Pediatric inpatient ward in Suez Canal University Hospital selected through a simple random sample. History taking as well as complete stool analysis and enzyme immunoassay to determine toxins A and B of *C. difficile* in stool samples were done. *Results:* Determination of toxins A and B of *C. difficile* in stool samples was found positive in 8.9% of the studied sample. Prevalence of *C. difficile* was slightly higher in infants than in children and in males more than in females. All 8 patients (100%) with *Clostridium difficile* infection (CDI) presented by diarrhea, 25% of them presented by abdominal pain and none of them presented by bloody stool. *C. difficile* infection was highest among patients having Cephalosporin (18.2%) followed by combination therapy between penicillin and cephalosporin (8.6%) while the lowest prevalence was among those on Penicillins only (4.8%). *Conclusion: C. difficile* infection is prevalent in hospitalized children receiving antibiotics therapy causing a wide variety of GIT symptoms mainly diarrhea.**

**Keywords:** *Clostridium difficile, antibiotic-associated diarrhea*

## Introduction

Clostridium difficile (*C. difficile*), a Gram-positive spore-forming bacillus, is the most common identifiable etiologic agent of antibiotic-associated diarrhea[(1,2)]. Initially described as a member of the commensal bacteria of neonates[(3)], *C. difficile* was identified as a causal agent of antibiotic-associated diarrhea in the 1970s[(4)]. The clinical presentation of Clostridium difficile-associated disease (CDAD) can range from asymptomatic carriage in the gastrointestinal tract, mild diarrhea, and potentially fatal pseudomembranous colitis[(1,2)]. Symptoms occur secondary to the production of two exotoxins, toxin A, primarily an enterotoxin and toxin B, acytotoxin. Both disrupt the integrity of the colonic mucosa[(5)]. Clostridium difficile is the most common cause of healthcare associated diarrhea in the United States, with significant associated morbidity, mortality, and healthcare costs[(6)]. The rate and severity of CDI have

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been increasing among children[7]. Much of this changing epidemiology has been attributed to the emergence of a hypervirulent strain of *C. difficile*, referred to as the North American pulsed-field gel electrophoresis type 1, or NAP1 strain[8-10]. Traditionally, *C. difficile* was believed to cause less significant disease among children than among adults, although severe CDI in children has been reported[11]. Still, the epidemiology of severe CDI in children remains undefined[8]. *C. difficile* has been recognized as the most important nosocomial pathogen in adults who manifest gastrointestinal symptoms subsequent to the use of broad spectrum antibiotics [5].Clinical symptoms vary widely, from asymptomatic colonization to pseudomembranous colitis with bloody diarrhea, fever, and severe abdominal pain[12,13]. Nowadays, *C. difficile* can be an important cause of pediatric diarrhea[14,15]. The extent and degree of illness seem to be worse in children than in adults, e.g. in fulminant enterocolitis[16-18]. Diarrheal disease due to *C. difficile* has become an important public health concern especially with increases in the number of cases, the severity of disease, and documented instances of failures to therapy[19,20]. Changes in the composition of the intestinal flora have been implicated in the initiation or maintenance of CDAD, which occurs predominantly in patients whose colonic flora has been disrupted by antibiotic therapy[13,21]. It has been established that the use of antibiotics by children presents the same risk as for adults. However, most literature in this field stems from the collection and interpretation of data from developed countries, where the use of antibiotics is under rigid control. Data collected in developing countries, on the other hand, can lead to a different interpretation, due to the widespread use of antibiotics[13]. The present study aimed to assess the frequency of *C. difficile* infection in hospitalized children in Suez Canal University Hospital. The study evaluated also some other predicted contributing factors to *C. difficile* infection in hospitalized children as age, sex and the use of antibiotics.

## Materials and Methods

This study was conducted as a cross-sectional study to detect *C. difficile* infection in hospitalized children in Suez Canal University hospital between December 2012 and June 2013. The study involved 90 hospitalized children in the Pediatric ward in Suez Canal University Hospital selected through a simple random sample. Patients were included in the study according to the following inclusion and exclusion criteria: *Inclusion criteria:* 1) Hospitalized patients for 3 days or more with diarrhea. 2) Receiving anti-biotic therapy. 3) Age between 1 month and 6 years. 4) Both genders. *Exclusion criteria:* 1) Patients who have been already diagnosed for other causes of diarrhea. 2) Patients with multiple pathogenic causes including CDAD are excluded. History was taken from patients mothers including: 1) Personal history (name, age, sex, address, etc), 2) Full medical history with emphasis on gastrointestinal manifestations especially diarrhea, abdominal pain, and presence of bloody stool. Diarrhea was defined as three or more loose stool that takes the shape of the container[22], 3) History of hospital admission in the last 3 months. 4) History of drug intake during hospitalization and prior to hospital admission especially antibiotics.

*Laboratory investigations*

Complete stool analysis to detect presence of bloody stool and to exclude other causes of diarrhea. Determination of toxins A and B of *C. difficile* in stool sample by an enzyme linked immunosorbent assay (ELISA) kit (Ridascreen Clostridium difficile

Toxin A/B" from R-Biopharm AG, Darmstadt, Germany).

## Results

Table (1) illustrates the demographic data of the studied children. Children "2–6 years" were slightly more represented in the studied sample (55.6%) than infants "1 month to 2 years" (44.4%). Females were almost equally represented in the sample (52.3%) as males (47.8%). Prevalence of *C. difficile* was 8.9% (8 out of 90) of the studied children (Figure 1). It was slightly higher among infants (9 infants, 10%) than children (7 children, 8%). The prevalence of *C. difficile* was higher among males (11.6%) than females (6.4%) (Table 3).

**Table 1:** Socio-demographic data of the studied population

| Socio-demographic data | No. (n=90) | % |
|---|---|---|
| Age | | |
| *Infant* | 40 | 44.4 |
| *Child* | 50 | 55.6 |
| Sex | | |
| *Male* | 43 | 47.8 |
| *Female* | 47 | 52.2 |

Infant (1 month - 2 years), Child (2 -6 years)

Table (2) illustrates that 16.7% of the children suffered from diarrhea and only 2.2% of them suffered from abdominal pain. None of the studied children had bloody stools. All 8 patients (100%) with CDI presented by diarrhea, 25% of them were presented by abdominal pain and none of them were presented by bloody stool (Fig. 2). *C. difficile* infection was highest among patients receiving Cephalosporin (18.2%) followed by combination therapy of penicillin and cephalosporin (8.6%) while the lowest prevalence was among those on Penicillins only (4.8%) (Tables 4,5). Antibiotics were received during hospitalization, and doses varied according to body weight.

**Table 2:** *C. difficile* infection and demographic data of the studied population

| Demographic data | *C .difficile* infection | | *p* value* |
|---|---|---|---|
| | Positive (n=8) | Negative (n=82) | |
| Age | | | |
| Infant (n=40) | 4 (10.0 %) | 36 (90.0 %) | 0.51 |
| Child (n=50) | 4 (8.0 %) | 46 (92.0 %) | |
| Sex | | | |
| Male (n=43) | 5 (11.6 %) | 38 (88.4 %) | 0.31 |
| Female (n=47) | 3 (6.4 %) | 44 (93.6 %) | |

**p* <0.05 is considered significant, using t-test

## Discussion

The study included 90 hospitalized children in the Pediatric ward at Suez Canal University Hospital. Stool samples were collected to perform complete stool analysis (data not shown) and determination of toxins A and B of *C. difficile* in stool sample by an enzyme immunoassay. In the present study, Children were slightly more represented in the studied sample (55.6%) than infants (44.4%). Prevalence of *C. difficile*

was almost equal among infants and children being slightly higher among infants (10% of infants) than children (8% of children), and the difference in *C. difficile* prevalence was statistically insignificant according to age. This was not supported by Smathers et al.( 2008)[23] who conducted a retrospective cohort study in the United States of America. The analysis involved 13.7 million inpatients over the 5-year study period from 2005 to 2009. The median age of participants was 5 years old and the median age for children with CDAD was 3 years old. Another study conducted in United States of America involved 82 children with CDAD was done and the median age of children with severe CDI (48 patients) was 5.9 years old. However, the median age of children with non-severe CDI (32 patients) was1.8 years old and the difference in *C. difficile* prevalence was statistically significant according to age[15]. Females were almost equally represented in the sample (52.3%) compared to males (47.8%). On the other hand, the prevalence of *C. difficile* was higher among males (11.6%) than females (6.4%) showing no statistical significant difference as regards sex.

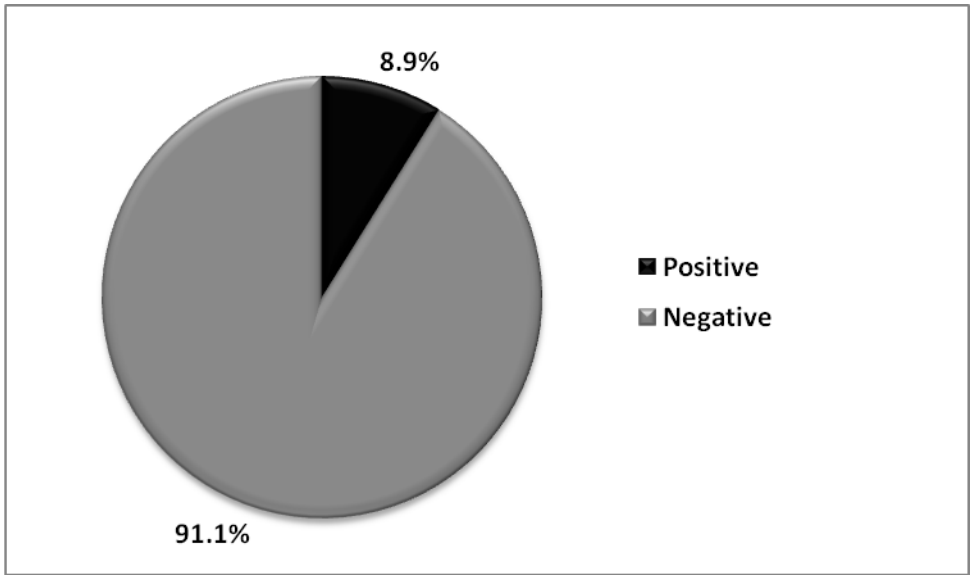


**Figure 1:** Distribution of *C. difficile* toxins in stool of the studied children

**Table 3:** Clinical presentation of study patients

| GIT manifestations | No. | % |
|---|---|---|
| Diarrhea | 15 | 16.7 |
| Abdominal pain | 2 | 2.2 |
| Bloody stool | 0 | 0 |
| No manifestations | 73 | 81.1 |

**Table 4:** Antibiotic use of the study population

| Antibiotic | Frequency | % |
|---|---|---|
| Penicillins | 21 | 23.3 |
| Cephalosporin | 11 | 12.2 |
| Combination (penicillin + cephalosporin) | 58 | 64.4 |

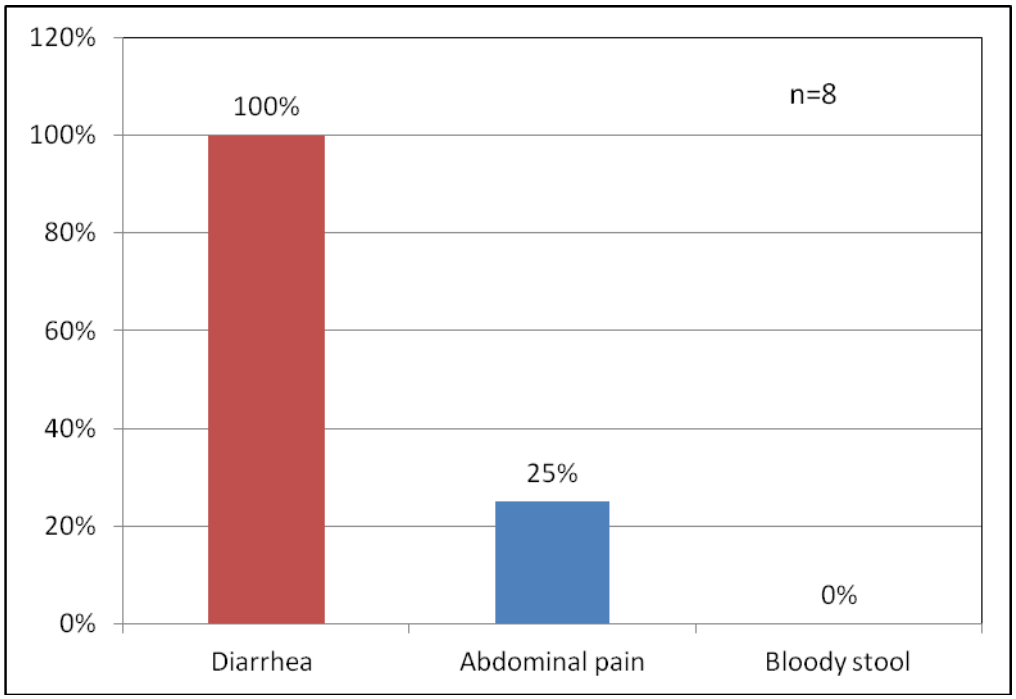


**Figure 2:** Clinical presentation among children with *C. difficile* infection

This was supported by Smathers et al. (2008)[23], as boys accounted for 54% of the total cases with CDAD and the difference in *C. difficile* prevalence was statistically insignificant according to sex. Kim et al. (2012)[15] documented that boys accounted for 48% of cases with both severe and non-severe CDI but without statistical significance according to sex.

**Table 5:** Relation between *C. difficile* infection and type of antibiotic used

| Antibiotics | *C. difficile* infection | | | *p** |
|---|---|---|---|---|
| | Positive (n=8) | Negative (n=82) | Total (n=90) | 0.48 |
| Penicillins | 1 (4.8 %) | 20 (95.2 %) | 21 (100 %) | |
| Cephalosporin | 2 (18.2 %) | 9 (81.8 %) | 11 (100 %) | |
| Combination(Penicillin+ Cephalosporin) | 5 (8.6 %) | 53 (91.4 %) | 58 (100 %) | |
| Total | 8 (8.9 %) | 82 (91.1 %) | 90 (100 %) | |

**p* <0.05 is considered significant, using Chi-square test

The same was reported by Khanna and Mellow (2012)[24], as there were 46,176 cases of CDI, and 48.1% of them were females. In the present study, determination of toxins A and B of *C. difficile* in stool sample was done by an enzyme immunoassay. *C. difficile* toxins were found positive in 8.9% of the studied children. This was supported by Kling (2013)[25], a cohort study; conducted on 20 European countries. A total of 3923 fecal samples from 482 participating hospitals were tested for membrane enzyme immunoassays for glutamate dehydrogenase and for toxins A and B. The mean positivity rate was 8.8%. Pnik et al. (2012)[26] conducted another cross-sectional study in Brazil. *C. difficiles* strains were detected in 14 of 210 (6.7%) stool samples from children in Riode Janeiro, stool samples were first cultured on selective media

then isolated bacteria was tested for toxin production by enzyme immunoassay and confirmed by PCR for presence of toxin A gene. On the other hand, in Middle East, Shakiee et al. (2012)[15], conducted across-sectional study in Jordan, found a 9.7% prevalence rate of *C. difficile* or its toxin in stools of hospitalized children. Toxin detection was done by enzyme immunoassay However, Wultańska et al. (2010)[27] conducted a cross-sectional study in Poland. Stool samples were examined for the presence of toxin A/B of *C. difficile* by ELISA and toxigenicity of strains was confirmed using PCR. The percentage of children infected with *C. difficile* was 68.6%, toxigenic of *C. difficile* strains (toxin A positive, toxin B positive and CDT negative) accounted for 35%, (toxin A negative, toxin B positive and CDT negative) accounted for 10% ,and 5% of infected children were strains of (toxin A positive, toxin B positive and CDT positive). 50% of the cultivated strains were non-toxigenic. In the present study, all the patients who had tested positive for *C. difficile* toxins in stool (n=8) presented by gastrointestinal symptoms in the form of diarrhea in 100% of them, abdominal pain in 25%. However, none of them had bloody stool. This was supported by (Pepin et al., 2004)[10], as 75% of patient with CDI presented by diarrhea. Pepin found that the relation between *C. difficile* infection and each diarrhea and abdominal pain is statistically significant. In the present study, all included patients in study population were on antibiotic treatment. *C. difficile* infection was highest among patients having Cephalosporin (18.2%) followed by combination therapy between penicillin and cephalosporin (8.6%) while the lowest prevalence was among those on Penicillin (4.8%). However, there was no significant relation between type of antibiotics used and *C. difficile* infection. This was supported by Shaklee et al., (2012)[15] who concluded that exposure to multiple antibiotic classes were risk factors for severe CDI. Another retrospective cohort study was conducted by King et al. (2011)[28] on 115 patients with CDI reported that 91 patients (79.1%) received at least one dose of an antimicrobial agent during admission. 35 patients (39%) received only one antibiotic, and 28 (31%) received two antibiotics. Fifteen patients (16%) received three antibiotics, and 13 patients (14%) received four or more antibiotics. Patients who received at least one dose of an antibiotic were at greater risk for developing CDI than were those who did not receive antimicrobial therapy. Moreover, Kelly and Lamont (2008)[12] through their cohort retrospective study concluded that the majority of cases of CDAD (67%) occurred in children with complex chronic conditions. The complex chronic conditions might have increased the risk for CDAD because of increased antibiotic exposure and longer hospitalizations.

The main limitation in this present study was the small sample size. Moreover, lack of further investigations to exclude other pathogens with similar clinical presentation due to the limitation of resources. On the other hand, patients who have been tested positive by ELISA and manifested clinically by diarrhea were not confirmed by further investigations as real time PCR and endoscopy. In addition, the patients with CDI included in the study were all inpatients, and we did not include the patients diagnosed after discharge or who developed CDI during admission. In addition, the duration of the antimicrobial therapy was not analyzed.

## Conclusion

We concluded that *C. difficile* infection is prevalent among hospitalized children receiving anti-biotic therapy causing a wide variety of GIT symptoms mainly diarrhea.

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