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Clostridium difficile Colonization Before and After Hospitalization in Children

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What is already known on this topic?

- *The most important risk factor for Clostridium difficile infection is antibiotic exposure and hospitalization.*
- *Recent studies have shown growing numbers of community-acquired infection cases as well.*

What this study adds on this topic?

- *This study is the first study investigating the prevalence of toxigenic C. difficile colonization in hospitalized children in our country.*
- *C. difficile positivity is high in children on the first day of hospitalization in the third-level hospitals.*

ABSTRACT

Background: Beginning in the early 2000s, *Clostridium difficile* infection has become a major health problem in the United States, Canada, and in most European countries and has not only increased in incidence but also the severity. There are 2 conditions for the development of *C. difficile* infection: disruption of the normal gastrointestinal flora, and exogenous ingestion of the microorganism. We aimed to study *C. difficile* colonization in hospitalized children. We identified 2 issues: (1) the relationship between risks before hospital admission and colonization on the first day of hospitalization and (2) the effect of the factors that patients are exposed to during hospitalization on the colonization status at discharge.

Methods: Patients aged between 2 and 18 years who were hospitalized with various diagnoses were included in this study. *C. difficile* toxin A/B was investigated in the stool samples taken on the admission and discharge days.

Results: One hundred six patients were included in the study, of whom 24.5% and 48.1% of hemato-oncology patients were positive for *C. difficile* toxin A/B. Antibiotic usage within 1 month preceding hospitalization and the presence of underlying disease impact the *C. difficile* colonization status on the first day of hospitalization.

Conclusion: Toxigenic *C. difficile* colonization prevalence is high in hospitalized children, especially in the hemato-oncology patient group.

Keywords: Antibiotic, children, Clostridium difficile, colonization, toxin

INTRODUCTION

Clostridium difficile is a Gram-positive, anaerobic, toxin-producing, spore-forming enteric bacillus. It was first isolated in 1935 from the stool of healthy neonates.[1] *C. difficile* infection is a toxin-mediated intestinal disease, the symptoms of which can range from mild diarrhea to severe abdominal pain and fever. The clinical outcomes can alternate from asymptomatic colonization to the formation of pseudomembranes in the colon or toxic megacolon, bowel perforation, sepsis, shock, and death.[2]

C. difficile has become a major health problem in the United States, Canada, and in most European countries in recent years. The epidemiology of *C. difficile* infection changed dramatically in the early 2000s. The disease not only increased in incidence but also severity.[3] Its incidence increased from 24 per 10 000 discharges to 58 per 10 000 discharges ($P < .001$) among hospitalized children in the United States during 2003-2012.[4] There has also been an increase in the rate of *C. difficile* infection in some chronic diseases (cystic fibrosis, inflammatory bowel disease, solid organ transplant, human immunodeficiency virus infection, hematopoietic stem

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cell transplantation, neoplastic disease, and pancreatitis).[4] The most important risk factor for *C. difficile* infection is antibiotic exposure.[5] The other well-known risk factors are gastric acid suppression, co-morbid conditions such as neuromuscular, cardiovascular, respiratory, renal, gastrointestinal, hematologic, immunologic, metabolic, and malignant diseases, and congenital disorders.[4,6,7] Hospitalization is another risk factor for *C. difficile* infection.[8] However, recent studies have shown growing numbers of community-acquired *C. difficile* infection cases as well.[9-11]

In this study, we investigated the prevalence of toxigenic *C. difficile*. We aimed to determine the prevalence of *C. difficile* colonization in children on the first day of hospitalization and investigate the relationship between age, gender, presence of underlying diseases, and antibiotic therapy before hospitalization. We also investigated the effect of antibiotic treatment and length of stay in patients who were *C. difficile* -negative at admission.

METHODS

Population and Sample

Patients aged between 2 and 18 years who were hospitalized with various diagnoses in Istanbul University-Cerrahpaşa, Cerrahpasa Medical Faculty Department of Pediatrics between June 2015 and February 2016 were included in this study. Ethics committee approval was obtained for the study from Istanbul University-Cerrahpasa (Faculty Deanship Clinical Research Ethics Committee, Date: June 5, 2015; Number: 29430533-903.99-141904). Written informed consent has been obtained from the patient's parents. Stool samples were taken from the patients on the first day of hospitalization and *C. difficile* toxin A/B was investigated. *C. difficile* toxin A/B was investigated by taking a second stool sample before discharge from patients who were found to be *C. difficile* toxin A/B-negative at admission and had received antibiotic treatment during hospitalization (Figure 1).

Data Collection

The patient's parents were interviewed face-to-face on the first day of hospitalization. Information was obtained on whether the patients had underlying diseases and had used antibiotics during the month preceding hospitalization. Informed consent of the parents was obtained. Hospital electronic medical records, patient files, and nurse records were also investigated.

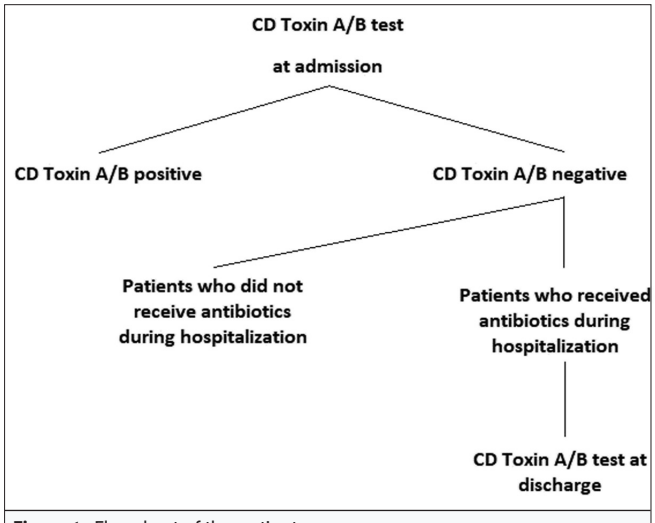

Figure 1. Flowchart of the patients.

Diagnostic Procedures

Collected stools were studied following the testing procedure using RIDASCREEN *C. difficile* Toxin A/B (C0801) kits. The cut-off was calculated as negative control+0.15. Values greater than 10% were considered positive.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM SPSS Corp.; Armonk, NY, USA) program was used for statistical analysis. Number (n) and percentage (%) values were used in the expression of descriptive data. When examining between-group differences for categorical variables with an expected value less than 5 in less than 20% of cells, a Pearson chi-square test was used. In the comparison of continuous variables, non-normally distributed data were expressed as the median, mean, and standard deviation values and the Mann–Whitney *U*-test was used. A value of $P < .05$ was determined as the statistical significance level.

RESULTS

Demographic Variables

One hundred six patients were included in the study. The average age of the patients was calculated as 7.3 years. The female/male ratio of the patients was 64/42.

Evaluation of the Results on the First Day of Hospitalization

There was no statistically significant difference between positive and negative patients in terms of age ($P = .791$; Mann–Whitney *U*-test). There was no significant difference between male and female patients at admission in terms of toxin positivity ($P = .433$; Pearson chi-square test) (Table 1). Stool samples were taken from each patient during hospitalization and *C. difficile* toxin A/B was found positive in 26 (24.5%) patients. Of the 106 patients included in our study, 54 had an underlying chronic disease. *C. difficile* toxin positivity was found to be significantly higher in patients with underlying disease ($P = .009$; Pearson chi-square test) (Table 1). The diagnoses of chronic patients and the number of *C. difficile* toxin A/B positive patients at hospitalization are presented in Table 2. Since the number of subjects was not sufficient, statistical comparison between groups was not possible. The prevalence of *C. difficile* positivity was significantly higher in patients who had used antibiotics one month before hospitalization compared to the group without prior antibiotic use ($P = .01$; Pearson chi-square test) (Table 1). There was no difference in *C. difficile* positivity between patients who received a single antibiotic and those who received 2 or more antibiotics ($P = .679$; Pearson chi-square test) (Table 1). Antibiotic types and *C. difficile* positivity in patients receiving single antibiotic therapy are presented in Table 3. It was not possible to draw statistical conclusions from the distribution. The patients included in the study were hospitalized in 10 different services: hemato-oncology, infectious diseases, gastroenterology, nephrology, cardiology, neurology, rheumatology, endocrinology, metabolic diseases, and genetic diseases (Table 4). Most of the patients and positive results were distributed in the infectious diseases and hemato-oncology services. When

Table 1. Comparison of Demographic and Clinical Characteristics of the Groups

	***Clostridium difficile* Toxin A/B Positive Patients, n = 26**	***Clostridium difficile* Toxin A/B Negative Patients, n = 80**	***P***	
Gender, n (%)				
Male	14 (21.9)	50 (78.1)	0.433	χ^{2**}
Female	12 (28.9)	30 (71.1)		
Presence of underlying disease, n (%)				
Yes	19 (35.2)	35 (64.8)	0.009	χ^2
No	7 (13.5)	45 (86.5)		
Antibiotic usage within one month before hospitalization, n (%)				
Yes	23 (31.9)	49 (68.1)	0.01	χ^2
No	3 (8.8)	31 (91.2)		
Number of antibiotics, n (%)				
Single antibiotic use	11 (29.7)	26 (70.3)	0.679	χ^2
Multiple antibiotic use	12 (34.3)	23 (65.7)		
Department, n (%)				
Hemato-Oncology	13 (48.1)	14 (51.9)	0.01	χ^2
Infectious diseases	6 (13.6)	38 (86.4)		

**Pearson chi-square test.

Table 2. Diagnoses of Patients with Underlying Disease and Positive *Clostridium difficile* Toxin A/B Results

	Diagnosis	**Number of Patients, (N = 54)**	**Number of *Clostridium difficile* Positive Patients, (N = 19)**
1	Acute lymphoblastic leukemia	11	5
2	Osteosarcoma	4	2
3	Neuroblastoma	3	2
4	Acute myeloblastic leukemia	2	2
5	Lymphoma	2	0
6	Ewing sarcoma	1	1
7	Wilms tumor	1	1
8	Retinoblastoma	1	0
9	Medulloblastoma	1	0
10	Rhabdomyosarcoma	1	0
11	Systemic lupus erythematosus	2	1
12	Wegener's granulomatosis	1	0
13	Juvenile rheumatoid arthritis	1	0
14	Autoimmune encephalitis	1	1
15	Cystic fibrosis	5	1
16	Bronchiectasis	1	0
17	Neutrophil dysfunction	1	0
18	Chronic granulomatous disease	2	0
19	Chronic mucocutaneous candidiasis	1	0
20	Agammaglobulinemia	2	0
21	Liver transplantation	1	0
22	Post-transplant lymphoproliferative disease	1	0
23	Ulcerative colitis	1	0
24	Neurointestinal dysplasia	1	0
25	Intestinal tuberculosis	1	0
26	Food allergy	1	1
27	Ornithine transcarbamoylase deficiency	1	1
28	Chronic renal failure	1	0
29	Nephrotic syndrome	1	0
30	Meningomyelocele	1	1

Table 3. Antibiotic Type and Positive *Clostridium difficile* Toxin A/B Results in Patients Receiving Single Antibiotic Therapy

	Antibiotic	Number of Patients, (N = 37)	Number of Positive Results, (N = 11)
1	Cefuroxime	13	5
2	Amoxicillin clavulanic acid	9	1
3	Meronem	2	1
4	Gentamicin	1	1
5	Ceftriaxone	1	1
6	Azithromycin	1	1
7	Cefazole	1	1
8	Ciprofloxacin	2	0
9	Amikacin	1	0
10	Amoxicillin	1	0
11	Trimethoprim-sulfamethoxazole	1	0
12	Metronidazole	1	0
13	Fluconazole	1	0
14	Ampicillin sulbactam	1	0
15	Penicillin	1	0

these 2 services were compared, the prevalence of *C. difficile* at hospitalization in hemato-oncology patients was found to be statistically significantly higher ($P = .01$; Pearson chi-square test) (Table 1).

Evaluation of the Results on Discharge

From 49 patients who were found to be *C. difficile* Toxin A/B-negative on admission and were using antibiotics during their hospitalization, second samples were taken again at discharge, and positivity was detected in 3 patients (6.1%) after antibiotics. The mean hospital stay was 21.69±18.51 days and there was no statistically significant difference between positive and negative patients in terms of hospital stay ($P = .860$; Mann–Whitney *U*-test) (Table 5).

DISCUSSION

There are 2 conditions for the development of *C. difficile* infection: disruption of the normal gastrointestinal flora, reducing the colonization resistance in favor of *C. difficile*; and exogenous ingestion of the microorganism.[12] The major virulence factors of *C. difficile* are toxin A and toxin B; the toxin-negative strains are nonpathogenic. Toxins cause impairment in the intestinal epithelium and are encoded by the genes *tcdA* and *tcdB* located at the pathogenicity locus. The toxin A and/or B enzyme immunoassay (EIA) test for stool is widely used in the diagnosis of *C. difficile* infection. Testing for both toxins was recommended by the Committee on Infectious Disease of the American Academy of Pediatrics. This is due to the physiological fluctuations of *C. difficile* toxin production during disease which means that the toxin B assay alone failed to identify as much as 34.9% of infection cases.[13]

Table 4. Number of Patients and Positive *Clostridium difficile* Toxin A/B Results by Service

1	Infection	27	13
2	Hemato-Oncology	44	6
3	Neurology	9	2
4	Cardiology	8	1
5	Gastroenterology	5	1
6	Metabolic diseases	1	1
7	Rheumatology	4	1
8	Nephrology	3	1
9	Endocrinology	4	0
10	Genetic diseases	1	0

In our study, the presence of *C. difficile* toxin A/B was investigated by taking stool samples during hospitalization regardless of the clinical findings, diagnoses, and complaints of the patients, and the prevalence of *C. difficile* colonization in hospitalized children was found to be 24.5%. Zacharioudakis et al.[14] conducted a systematic review and meta-analysis of 19 studies in which the prevalence of *C. difficile* in hospitalizations was investigated and 8725 patients were included, and they found that the prevalence of toxigenic *C. difficile* colonization was 8.1% and showed a tendency to increase over time. Furuichi et al.[15] investigated *C. difficile* colonization in hospitalized children in Japan in 2012-2013. Two hundred fifty-one patients were divided into 2 groups according to the presence of underlying disease and the frequency of *C. difficile* and the toxin-producing *C. difficile* were 21.6% and 9.0% respectively in children without underlying diseases. In patients with underlying disease, these rates were found to be 30.8% and 23.1%, respectively, higher than in the first group. Colonization with *C. difficile* does not require treatment as it is not believed to be a direct precursor for *C. difficile* infection. On the other hand, a *C. difficile* infection is a clear indication that therapy is required.[16]

The importance of *C. difficile* colonization is not a well-understood subject. The colonization rate of *C. difficile* in newborns and infants younger than 2 years ranges from 2.5% to 90%.[17] Zacharioudakis et al.[14] showed that patients

Table 5. Length of Stay in Hospital and *Clostridium difficile* Toxin Positivity

	Length of Stay in Hospital (Days)		
***C. difficile* Toxin**	**Mean ± SD**	**Median**	***P*****
Positive	16.33 ± 1.15	17.00	.860
Negative	22.04 ± 19.06	17.00	
Total	21.69 ± 18.51	17.00	

**Mann–Whitney *U*-test.

colonized with *C. difficile* during hospitalization have a 5.9-fold higher risk of *C. difficile* infection compared to non-colonized patients. Recent studies show that asymptomatic carriers may be involved in transmission.[18] In a recent meta-analysis of *C. difficile* colonization at hospital admission, the risk of developing *C. difficile* infection during hospital stay was 18.4%.[14] Patients with asymptomatic colonization, whose colonization status is uncertain, may cause horizontal transmission inside the health care unit.[19] *C. difficile* is transmitted by the fecal-oral route of ingestion of microorganisms. Its spores can be taken from patients with colonization as a result of contact, and are often transported through the hands of hospital staff. Patients staying in the same room with *C. difficile* -positive patients in the hospital get infected with *C. difficile* in a shorter time than people who are in a single room or whose roommate is negative for *C. difficile* (3.2 days vs 18.9 days).[20]

Recent antibiotic exposure is believed to be the most important risk factor for the development of *C. difficile* infection in both adults and children.[13] Our study also supports this relationship. The prevalence of *C. difficile* positivity was significantly higher in patients who had antibiotic use before hospitalization compared to the group without prior antibiotic use ($P = .01$).

In this study, we found a 48.1% *C. difficile* positivity in pediatric hematology-oncology patients. The study, conducted in a 566-bed academic medical care center, showed that the prevalence of colonization with toxigenic *C. difficile* among adult patients with hematological malignancies and/or bone marrow transplant at admission was 9.3%, with 13.3% of the colonized patients developing the symptoms during hospitalization.[21] Immunocompromised (IMC) patients seem to be at serious risk of recurrent *C. difficile* infections. A recent study looking at the results of 149 IMC patients (62 hematological conditions, 36 solid organ transplants, 13 receiving high-dose prednisone, 38 undergoing chemotherapy) showed that the rate of recurrent *C. difficile* infection was significantly higher (OR 2.7, 95% CI 1.6-5).[22]

In the adult hematology-oncology population, *C. difficile* infection rates range from 6% to 33%, with most cases happening early post-transplantation (within 30 days). This incidence rate is higher than the *C. difficile* infection rate of the general population, which is 1%.[23]

In a retrospective study in Turkey, 986 children with diarrhea were investigated for *C. difficile* infection and 100 children were diagnosed with *C. difficile* infection among 12 196 hospitalized children during 2012-2014.[24] The presence of underlying chronic diseases, presence of solid organ tumors, and hospitalization in the hematology and oncology ward were found to be independent risk factors for *C. difficile* infection. This study is the first study in Turkey investigating the prevalence of toxigenic *C. difficile* in hospitalized children. Malignancy, chemotherapy, and long-term use of broad-spectrum antibiotics predispose hemato-oncology patients to *C. difficile*. These conditions can cause *C. difficile* transmission or lead to progression from asymptomatic colonization to severe disease.[25,26] For this reason, it is important to determine the index cases by searching for *C. difficile*-positive patients and to separate them from patients with risk factors and suppressed immunity in terms of controlling the disease.

Diagnostic Limitations

In our study, we investigated the prevalence of *C. difficile* colonization and the risk factors affecting it. We did not investigate the effect of this colonization on the clinical condition of the patients, their complaints, and symptoms. The small number of patients is the most significant limitation of this study. We believe that further studies should be conducted in the pediatric age group, and the importance of colonization, especially in the hemato-oncology patient group, should be investigated further.

CONCLUSION

Toxigenic *C. difficile* colonization on the first day of hospitalization is high in children. The presence of underlying disease and the history of antibiotic usage within one month preceding hospitalization were associated with this. In hemato-oncology patients, colonization rates reach 48.1%. The effects of such high toxigenic *C. difficile* colonization rates in IMC patients need to be investigated in further studies with a larger sample size.

Ethical Committee Approval: Ethics committee approval was received for the study from Istanbul University-Cerrahpasa (Faculty Deanship Clinical Research Ethics Committee, Date: June 5, 2015; Number: 29430533-903.99-141904).

Informed Consent: Written informed consent has been obtained from the patient's parents.

Peer Review: Externally peer-reviewed.

Author Contributions: Concept – T.R., T.K.; Design – T.R., T.K.; Supervision – T.E., T.K.; Materials – T.R., P.Y.; Data Collection and/or Processing – T.R., P.Y., B.K.; Analysis and/or Interpretation – T.R., P.Y., B.K.; Literature Review – T.R., T.E., T.K.; Writing – T.R., P.Y.; Critical Review – T.K.

Conflict of Interest: The authors have no conflicts of interest to declare.

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