

Clostridium difficile infection in children: characteristics and treatment – a single-center study from Romania

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The incidence of Clostridium difficile infection (CDI) in children is progressively increasing. The study evaluated the characteristics and antibacterial treatment of CDI at a Romanian pediatric gastroenterology department. We performed a retrospective study to analyze cases diagnosed with CDI, identified through immunoassays for Clostridium difficile toxins in stools, between January 1, 2005 and December 31, 2015. Eighty-nine episodes of CDI were diagnosed in 73 patients. We noticed an increasing incidence reaching maximum in 2014 with 6.9 cases/1000 patients. Almost 40% of patients had community-acquired CDI. The most frequently associated comorbidities were inflammatory bowel disease and cow's milk allergy. There was a small percentage of recurrent episodes (24.2%). Metronidazole was administered as first-line treatment in 49.2% of mild/moderate cases and proved effective in 79.4% of these. More than 70% of all patients in which metronidazole was not efficient had comorbidities, compared to 22.2% of patients where metronidazole was efficacious. The alternative was vancomycin which cured the disease in all cases. In severe forms, a combination of intravenous metronidazole and oral vancomycin was the efficient solution. Oral vancomycin was the efficacious treatment for the first recurrence. We report an increasing incidence of CDI in Romanian children. The failure rate for metronidazole treatment was low, thus metronidazole may be safely recommended for the first episode of mild/moderate CDI. Vancomycin proved effective in all cases, regardless of the first episode or recurrence, and may be used efficiently as first-line treatment.

Key words: *Clostridium difficile*; antibacterial treatment; children

INTRODUCTION

Clostridium difficile is an anaerobe, gram-positive, spore-forming bacillus which was identified in 1935 in fecal flora of newborns, but was considered non-pathogenic for a long time (1). Years later, in 1978, Bartlett *et al.* isolated *Clostridium difficile* and proved it to be the source of cytotoxins in the stools of patients with pseudomembranous colitis (2). Lately, several studies demonstrated an increase in the incidence of this infection in adults. Although in the past *Clostridium difficile* infection (CDI) was not considered a problem in pediatric age, in the last decades several studies have been published demonstrating an increasing incidence of the disease in children in the USA and Europe (3-5). CDI has always been regarded as a nosocomial infection and is in-

deed the second most common hospital-acquired diarrheal disease after rotavirus infection (6). Use of antibiotics and prolonged hospital stay are considered risk factors for CDI. Associations between CDI and inflammatory bowel disease, immune deficiencies, Hirschsprung's disease, cystic fibrosis, hematologic malignancies, or organ transplantation have

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been reported. Several studies have been published demonstrating a significant percentage of community-acquired CDI in pediatric patients (7, 8). Therapeutic approach is difficult in the severe forms of the disease, especially in patients with comorbidities or in patients with recurrent CDI.

Just a small number of articles consider epidemiological aspects of CDI in Romania (9-11). Still, its incidence has recently been shown to increase in adult patients (12). We found no studies assessing the characteristics of CDI and therapeutic approach to pediatric CDI in our country. This study aimed to evaluate the characteristics and antibacterial treatment of CDI based on the experience of a single Romanian pediatric gastroenterology department.

PATIENTS AND METHODS

This retrospective study analyzed the cases diagnosed with CDI and admitted to the Pediatric Gastroenterology Department, Grigore Alexandrescu Emergency Children's Hospital, between January 1, 2005 and December 31, 2015. We selected cases identified through enzyme immunoassays for *Clostridium difficile* toxin A in stools between 2005 and 2007, and through enzyme immunoassays for *Clostridium difficile* toxins A and B between 2008 and 2015. The tests were initially performed in a laboratory outside our department and since 2014, they have become available in our clinical laboratory.

The following data were extracted from patient medical records: age, sex, past medical history, clinical manifestations, comorbidities, laboratory diagnostic tests, treatment of the first episode, recurrence of CDI, and treatment of recurrent episodes.

The antibiotic was considered efficacious when symptom relief and negative *Clostridium difficile* toxin test result were reported after treatment. A second CDI episode occurring after successful treatment of a previous, confirmed by positive test result, was considered a recurrence.

For statistical analysis, we used Microsoft Excel and SPSS v. 20. The χ^2 -test and Fisher test were used to compare categorical variables. The level of statistical significance was set at $p < 0.05$.

The study was approved by the Ethics Committee of the Grigore Alexandrescu Emergency Children's Hospital.

RESULTS

Between January 1, 2005 and December 31, 2015, 589 tests were performed in 404 patients. There were 89 episodes of CDI in 73 patients admitted to the Pediatric Gastroenterology Department, Grigore Alexandrescu Emergency Chil-

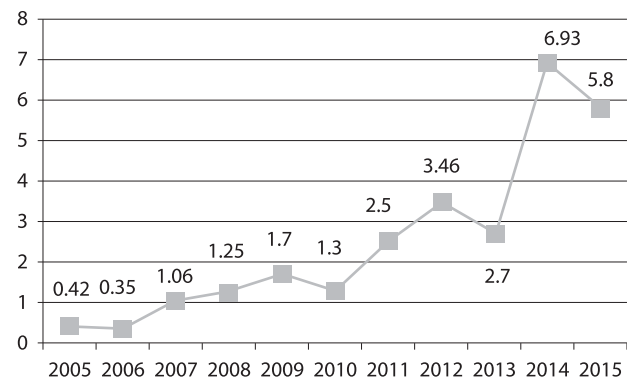


FIGURE 1. Incidence of *Clostridium difficile* infection/1000 patients admitted 2005-2015.

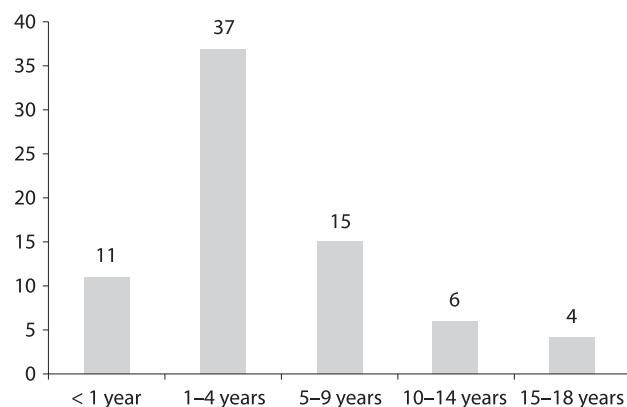


FIGURE 2. Distribution of patients with *Clostridium difficile* infection according to age.

dren's Hospital. The mean incidence of CDI was 2.49 cases per 1000 admitted patients. The number of confirmed cases had an increasing tendency, from 0.42 cases/1000 patients admitted in 2005, reaching 3.46 cases/1000 patients admitted in 2012, to the maximum of 6.93 in 2014 (Figure 1).

The median age of patients with CDI was 28 months, ranging from five months to 16 years and 8 months. A large number of cases (37 patients) were diagnosed in the age group one to 4 years (Figure 2).

All patients had diarrhea (liquid stools) and 32 (43.8%) patients had bloody stools. There were 67 cases of mild to moderate forms of CDI and six cases with severe forms, one of which was confirmed to be pseudomembranous colitis, confirmed by colonoscopy. Regarding community-acquired CDI, 28 (38.3%) cases were reported. The characteristics of CDI patients are shown in Table 1.

Thirty-five (47.9%) cases were associated with one or more comorbidities (Table 2). Seventeen patients had severe underlying comorbidities (Hirschsprung's disease, inflammatory bowel disease, cystic fibrosis or intractable diarrhea). Six children had immunodeficiencies, three were diagnosed with celiac disease and 13 with food allergies.

TABLE 1. Characteristics of patients with *Clostridium difficile* infection (CDI)

Median age (months)	28
Sex M/F (n)	31/42
Mild/moderate forms (n, %)	67 (91.7%)
Severe forms (n, %)	6 (8.3%)
Community-acquired CDI (n)	28

TABLE 2. Patients with *Clostridium difficile* infection – associated comorbidities

Comorbidity (N=35)	
Severe (n₁=17)	
Hirschsprung's disease (n)	5
Inflammatory bowel disease	10
Ulcerative colitis (n)	8
Crohn's disease (n)	1
Inflammatory bowel disease, type unclassified (n)	1
Cystic fibrosis	1
Intractable diarrhea	1
Immunodeficiencies (n₂=6)	
Ig A deficiency (n)	3
Transient hypogammaglobulinemia of infancy (n)	2
Secondary immunodeficiency (n)	1
Others (n₃=16)	
• Celiac disease	3
• Cow's milk allergy (n)	10
• Multiple food allergies (n)	3

TABLE 3. Comparison between metronidazole response group and failure group

	Response group (n ₁ =27)	Failure group (n ₂ =7)
Mean age (months)	34.6	43.9
Female (n)	16	3
Male (n)	11	4
Community-acquired CDI (n, %)	10 (37)	3 (42.8)
Hospital-acquired CDI (n, %)	17 (63)	4 (57.2)
Comorbidities (n, %)	6 (22.2)	5 (71.4)

CDI = *Clostridium difficile* infection

Out of the 73 patients initially included, seven were lost from follow up. Complete data on treatment, course of the disease and outcome were available for 66 patients. We report 16 cases of recurrent CDI (24.2% of 66 patients). Twelve of 16 children with recurrent CDI had comorbidities and eight of them had severe underlying medical illnesses (three cases with Hirschsprung's disease and five cases with inflammatory bowel disease), as compared with seven of 57

patients without recurrences that had severe underlying medical illnesses (p=0.0026).

Oral metronidazole was administered as first-line therapy for CDI in 34 (49.2%) cases with mild/moderate forms of the disease (Table 3). This treatment proved efficacious in 27 (79.4%) of 34 cases, resulting in cure in 41.9% of patients with CDI. Failure of metronidazole therapy was reported both in hospital-acquired infections (4/21 cases) and community-acquired infections (3/13 cases). More than 70% of all patients in which metronidazole was not efficacious had comorbidities, as compared with 22.2% of patients where metronidazole was efficacious.

For the treatment of the first episode in mild/moderate forms of CDI, vancomycin was efficacious in all 33 cases (in 25 cases it was used as first-line therapy, and in eight cases as second-line therapy after metronidazole treatment failure), proving to be more efficacious than metronidazole. In severe forms, the combination of intravenous metronidazole and oral vancomycin was used. This approach cured all 6 patients. Three patients had associated severe digestive disease, i.e. Hirschsprung's disease or inflammatory bowel disease. Oral vancomycin was successfully administered for the first recurrence. In three cases with a second recurrence, rifaximin was the chosen therapy. Consecutively, no recurrences were reported.

DISCUSSION

Our study showed a significant increase, more than 4-fold, of CDI cases diagnosed between 2010 and 2015, as compared to previous years 2005-2009. This increase in CDI incidence is consistent with similar results reported from European studies (13-15). At the same time, the higher number of confirmed cases may be a consequence of recent improvements in diagnostic technique; namely, more cases were tested for *Clostridium difficile* and tests for both toxins were used. The increase in the incidence observed in 2014 may also be attributed to the fact that the diagnosis could be readily established with the tests available in our hospital.

The majority of study patients (50.7%) were children aged one to four years. In a recently published study, Zilberberg *et al.* analyzed CDI among hospitalized children in the United States between 1997 and 2006, and observed that the highest incidence of CDI was reported in the 1- to 4-year age group (4). We report the peak incidence between one and two years of age (41.1% of cases), the same as Pai *et al.*, who have published an analysis of pediatric CDI cases diagnosed in a UK tertiary hospital (16). CDI was also diagnosed in infants: 15% in our study, a small percentage compared to 26% in the US report (3). The experts point to the fact that in this age group, the presence of *Clostridium difficile* in stool

is not always pathologic, as infants may sometimes exhibit asymptomatic carriage.

Most of our patients had mild to moderate forms of CDI, but six patients had a severe form of the disease; one case with pseudomembranous colitis was admitted to the Intensive Care Unit and required complex therapeutic approach.

In recent years, an increasing number of studies reporting increasing incidence of community-acquired CDI have been published. For instance, a population-based study of CDI in pediatric residents (aged 0-18 years) of Olmsted County, Minnesota, from 1991 through 2009, demonstrated that the majority of cases (75%) were community-acquired, and the incidence of community-acquired CDI increased 10.5-fold (17). In our study, 38.3% of patients had community-acquired CDI.

A quarter of patients had associated gastrointestinal comorbidities, as patients were recruited among those admitted to Gastroenterology Department. It is interesting to note that the most frequently associated pathology was inflammatory bowel disease and cow's milk allergy (13.7% both); similar findings have been reported by *Pai et al.* (16).

In our study, we had recurrent episodes in only 24.2% of cases, which is a small percentage compared to other pediatric studies (18). In adults, severe underlying disease has been suggested to be a risk factor for CDI recurrence (19). In our study, 17 of 73 (23.3%) patients had severe underlying comorbidities (Hirschsprung's disease, inflammatory bowel disease, cystic fibrosis or intractable diarrhea).

Half of the patients (49.2%) received oral metronidazole as first-line treatment for mild to moderate CDI, according to the recommendation recently formulated by the American Academy of Pediatrics and sustained by several studies (16, 20-22). This antibiotic is considered efficacious and relatively inexpensive. Metronidazole is highly absorbed, with low fecal concentration levels in patients with diarrhea. A few recent reports show increased failure rates (23-26), but in our study it proved inefficient in only 20.6% of cases. In a recent review, *Vardakas et al.* analyzed studies regarding CDI treatment in adults and found that treatment failure was 22.4% with metronidazole (27).

The hospital-acquired infection was not a risk factor for metronidazole treatment failure. A high percentage of patients for which metronidazole treatment proved inefficient had different associated comorbidities (Hirschsprung's disease and inflammatory bowel disease), as opposed to patients in which metronidazole treatment was efficacious (71.4% vs. 22.2%). This difference was not statistically significant because of the small number of patients enrolled, but is suggestive of the fact that comorbidities might represent risk factors for metronidazole treatment failure. A recent retro-

spective study that evaluated CDI treatment in adults identified diabetes mellitus and sepsis as risk factors for metronidazole treatment failure in CDI (25).

Vancomycin is highly efficacious, is not absorbed, and achieves high fecal concentrations. It proved efficacious in all mild/moderate cases in the first episode, regardless of whether it was initial treatment or treatment of an episode ineffectively treated initially with metronidazole. Comparing our results with the study by *Vardakas et al.*, who found the rate of treatment failure to be 14.2% with vancomycin (27)], we found this antibiotic treatment to be more efficient.

Although oral vancomycin proved more efficacious than metronidazole for mild/moderate forms of CDI, we found no statistically significant difference for initial cure, observation concordant with the review by *Dreconja et al.* that evaluated CDI treatment in adults (28).

All severe cases responded well to the combination of intravenous metronidazole and oral vancomycin, although we had five cases with severe comorbidities and one case that developed a severe form of pseudomembranous colitis. Accordingly, we consider that this antibiotic combination should be the recommended treatment for severe CDI in children.

Patients with the first recurrence received vancomycin, which proved efficacious in all cases. A second recurrence occurred in four patients; they received rifaximin, which proved effective. Rifaximin is a rifamycin-based non-systemic antibiotic and data regarding its use in CDI treatment come from adult studies. These showed it to be useful for mild/moderate CDI cases that are resistant to metronidazole or vancomycin (29, 30) and can be considered as an optional treatment for recurrences (31). Randomized trials are needed to confirm these findings in children.

Our study had some limitations, i.e. it was a retrospective study in a limited number of cases and presents the experience of a single pediatrics department. Nevertheless, it contains Romanian data on CDI and treatment outcome in children; to date, very few data have been reported on the issue from our geographical area.

CONCLUSIONS

This was the first Romanian study assessing the characteristics and antibiotic treatment of CDI in children. We report an increasing incidence of CDI in children. The highest incidence of CDI was found in the one to four years age group. One quarter of patients had associated comorbidities, the most frequent being inflammatory bowel disease and cow's milk allergy. In our country, the rate of failure of metronida-

zole therapy for CDI treatment was found to be low, therefore we consider it may still be safely recommended for the first mild/moderate episode of CDI in children. Vancomycin proved efficacious in all cases, regardless of whether it was the first episode or recurrence of CDI, and we consider it to be the treatment of choice for CDI in children at this time.

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Autori su popunili the *Unified Competing Interest form* na www.icmje.org/coi_disclosure.pdf (dostupno na zahtjev) obrazac i izjavljuju: nemaju potporu niti jedne organizacije za objavljeni rad; nemaju finansijsku potporu niti jedne organizacije koja bi mogla imati interes za objavu ovog rada u posljednje 3 godine; nemaju drugih veza ili aktivnosti koje bi mogle utjecati na objavljeni rad./All authors have completed the *Unified Competing Interest form* at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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SAŽETAK

Infekcija bakterijom *Clostridium difficile* u djece: značajke i liječenje – istraživanje u jednom centru u Rumunjskoj

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Incidencija infekcije bakterijom Clostridium difficile (CDI) u djece u progresivnom je porastu. U ovom istraživanju procijenjene su značajke i antibakterijska terapija CDI na jednom odjelu pedijatrijske gastroenterologije u Rumunjskoj. Provedeno je retrospektivno istraživanje u kojem su analizirani slučajevi CDI utvrđeni na osnovi imunoloških testova na toksine Clostridium difficile u stolici od 1. siječnja 2005. do 31. prosinca 2015. godine. U 73 bolesnika dijagnosticirano je 89 epizoda CDI. Zabilježena je rastuća incidencija CDI s najvišom stopom 2014. godine sa 6,9 slučajeva na 1000 bolesnika. CDI stečena u zajednici utvrđena je u gotovo 40% bolesnika. Najčešće pridružene istodobne bolesti bile su upalna crijevna bolest i alergija na kravlje mlijeko. Postotak ponovljenih epizoda bio je nizak (24,2%). Metronidazol kao terapija prvog izbora davao se u 49,2% blažih/umjerenih slučajeva i pokazao se učinkovitim u 79,4% njih. Istodobno prisutne bolesti zabilježene su u više od 70% bolesnika u kojih metronidazol nije bio učinkovit u usporedbi s 22,2% bolesnika kod kojih je metronidazol bio učinkovit. Alternativna terapija bio je vankomicin koji je izliječio bolest u svim slučajevima. U teškim oblicima bolesti kombinacija intravenskog metronidazola i peroralnog vankomicina pokazala se učinkovitim rješenjem. Peroralni vankomicin bio je učinkovit u liječenju prve ponovljene epizode bolesti. Ukazuje se na rastuću incidenciju CDI kod rumunjske djece. Stopa neuspješnog liječenja metronidazolom bila je niska pa se ovaj lijek može sa sigurnošću preporučiti za liječenje prve epizode blaže/umjerene CDI. Vankomicin se pokazao učinkovitim u svim slučajevima bez obzira na to radi li se o prvoj ili ponovljenoj epizodi bolesti i može se učinkovito primijeniti kao terapija prvog izbora.

Ključne riječi: *Clostridium difficile*; antibakterijska terapija; djeca