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Clostridium difficile infection in Polish pediatric outpatients with inflammatory bowel disease

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Abstract The prevalence of *Clostridium difficile* infection (CDI) in pediatric patients with inflammatory bowel disease (IBD) is still not sufficiently recognized. We assessed the prevalence of CDI and recurrences in outpatients with IBD. In addition, the influence of IBD therapy on CDI and antimicrobial susceptibility of the potentially causative *C. difficile* strains was assessed. This was a prospective, single-center, observational study. All specimens were obtained between January 2005 and January 2007 from the IBD outpatient service and screened for *C. difficile* and its toxins. *C. difficile* isolates were genotyped by PCR ribotyping. Diagnosis of Crohn's disease (CD) and ulcerative colitis (UC) was based on Porto criteria. Severity of disease was assessed using the Hyams scale (for Crohn's disease) and the Truelove–Witts scale (for ulcerative colitis). One hundred and forty-three fecal samples from 58 pediatric IBD patients (21 with Crohn's disease and 37 with ulcerative colitis) were screened. The risk of *C. difficile* infection was 60% and was independent of disease type (CD or UC) ($\chi 2=2.5821$, $df=3$, $p=0.4606$). About 17% of pediatric IBD patients experienced a recurrence of CDI. All *C. difficile* strains were susceptible to metronidazole, vancomycin and rifampin. A high prevalence of *C. difficile* infection and recurrences in pediatric outpatients with IBD was observed, independent of disease type. There was no significant correlation between *C. difficile* infection and IBD therapy. PCR ribotyping revealed *C. difficile* re-infection and relapses during episodes of IBD in pediatric outpatients.

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Introduction

Clostridium difficile (*C. difficile*) strains cause severe diarrhea and colitis in both adults and children [1, 2]. Clinically important *C. difficile* strains usually produce two toxins: toxin A (TcdA)—an enterotoxin—and toxin B (TcdB)—a cytotoxin. These toxins are the main virulence factors of *C. difficile*, responsible for symptoms of diarrhea and inflammation [3, 4]. The epidemiology of *C. difficile* infection (CDI) has changed during the past decade. Traditional risk factors for *C. difficile*-associated disease are use of broad-spectrum antibiotic, chemotherapy, age over 65 years, and long-term hospitalization [5]. It was recently demonstrated that the use of immunomodulators

and the presence of inflammatory bowel diseases (IBD) are important risk factors for the development of CDI [5–7].

The two major types of IBD are ulcerative colitis (UC) and Crohn's disease (CD). Patients with IBD are often hospitalized with worsening diarrhea, which is attributable to progression of their underlying disease. Whether infection plays a role in this process is ill-defined. Potential risk factors for the acquisition of *C. difficile* infection in IBD patients include drugs used in medical treatment (e.g., sulfasalazine), which might alter the intestinal flora and promote colonization, altered immune status possibly related to therapeutic agents, nutritional status and frequent hospitalizations [8]. IBD patients with colonic involvement exhibited a significant association with development of *C. difficile* infection, with both Crohn's disease and ulcerative colitis [6].

Clostridium difficile infection may be difficult to distinguish from an IBD onset or relapse, given the similar symptoms of diarrhea, abdominal pain, and low-grade fever. In addition, CDI might be more commonly community-acquired than hospital-acquired [6, 7, 9]. The frequency of *C. difficile* infection in IBD pediatric patients has not been established precisely, but seemed to be higher than in adults [10].

The aim of the study was to determine the prevalence of *C. difficile* infection in pediatric outpatients with active and inactive (in remission) IBD. Also, this study determined whether diarrhea episodes were due to *C. difficile* re-infection or relapse. In addition, the influence of treatment on *C. difficile* infection and the antimicrobial susceptibility of *C. difficile* strains were assessed.

Materials and methods

Patients and specimens

This was a prospective, single-center, observational study. All specimens were submitted and processed immediately from the out-patients service of the Department of Pediatric Gastroenterology and Nutrition in Warsaw, Poland between January 2005 and January 2007. We collected data on patient age and gender, antibiotic treatment and hospitalization within the 3 months before the time of *C. difficile* diagnosis. All drugs used by patients were recorded. Diagnosis of Crohn's disease and ulcerative colitis was based on clinical signs and symptoms combined with endoscopic, histological, and radiological results, which were interpreted according to the Porto criteria [11]. We used the modified Truelove and Witts activity index for ulcerative colitis and pediatrically modified Hyams activity index (PCDAI) for Crohn's disease. Active disease was defined as symptomatic UC

with a Truelove–Witts score greater than 4 points and symptomatic CD with a PCDAI score greater than 10 points. The Truelove–Witts activity score is partly based on the patient's symptoms (number of stools, presence of blood in the stool, fever), but also on laboratory data (hemoglobin level, ESR) and nutritional assessment. The Hyams activity score assesses patient's symptoms (abdominal pain, bowel habits, overall well-being), laboratory findings (ESR, hemoglobin level, albumin level), physical examination of abdomen, presence of perianal complications, extraintestinal manifestation, and nutritional assessment.

Clostridium difficile infection recurrences were defined on the basis of defecation frequency as perceived by the patient. Increases should at least last for two consecutive days and stools should become progressively looser or new signs of severe colitis should develop. Microbiological evidence of the presence of toxins of *C. difficile* and/or the actual presence of toxin-producing *C. difficile* strains after an initial CDI treatment response should be presented. The fecal samples from patients with an active or an inactive form of IBD were collected in sterile universal collectors and send to the diagnostic laboratory. Stools samples were investigated for enteropathogenic organisms such as toxigenic *Escherichia coli*, *Salmonella* spp., *Shigella* spp., rota- and adenoviruses (VIKIA ROTA ADENO KIT; bioMérieux, Marcy l'Etoile, France). Patients with IBD were treated with sulfasalazine or mesalazine, azathioprine, cyclosporine, steroids and infliximab according to accepted clinical protocols. Patients with *C. difficile* infection (TcdA/TcdB toxins and/or toxigenic strains detection) were treated with metronidazole for 10–14 days per one episode, which was the standard antimicrobial treatment protocol.

Diagnosis of *C. difficile* infection

Diagnosis of CDI was based on a positive stool enzyme immunoassay (EIA) and/or on the isolation of toxigenic *C. difficile* strains. Either or both of the *C. difficile* toxins TcdA/TcdB were detected in the fecal samples with an enzyme immunoassay (*C. difficile* TOX A/B II™; TechLab, Blacksburg, VA, USA). All fecal samples were inoculated after enrichment (by using an alcohol-shock procedure) onto selective Columbia Agar supplemented with cycloserine-cefoxitin and amphotericin B (CCCA medium; bioMérieux, Marcy l'Etoile, France) for detection of *C. difficile*. Plates were incubated in an anaerobic chamber (Forma Scientific, Marietta, GA, USA) at 37°C for 2 days [12]. The isolates were identified as *C. difficile* by the characteristic morphology of colonies, the specific horse odor, the yellow–green fluorescence under UV light (365 nm), Gram staining results, and biochemical tests (API 20A; bioMérieux, Marcy l'Etoile, France). For the detection

of *tcdA/tcdB* and binary toxin genes (*cdtA* and *cdtB*) genes, PCR was performed as described previously [12].

PCR ribotyping

Clostridium difficile isolates were typed by the PCR ribotyping methods described earlier [12]. Banding patterns were compared with those of the library of PCR ribotypes at the ARL, Cardiff [13].

Determination of antibiotic susceptibility

Minimal inhibitory concentration (MICs) for metronidazole (MZ), vancomycin (VA), ciprofloxacin (CI), gatifloxacin (GA), moxifloxacin (MX), and rifampin (RI) were determined by E-test (AB Biodisc, Solna, Sweden) according to the manufacturer's instructions, as described previously [12]. According to the Clinical and Laboratory Standard Institute (CLSI) recommendations (formerly NCCLS), antibiotic resistance was defined as follows: MIC≥32 mg/L for metronidazole, MIC≥32 mg/L for vancomycin, MIC≥ 4 mg/L for ciprofloxacin, MIC≥4 mg/L for gatifloxacin and MIC≥4 mg/L for moxifloxacin [14]. Rifampin resistance was defined as MIC≥32 mg/L in accordance with O'Connor et al. [15].

Statistical analyses

The Chi-squared test for independence was used to verify the hypothesis that infection *C. difficile* was independent of the type of illness. The gender proportions in groups were investigated by the Chi-squared test; for multiple comparisons LSD *post hoc* Fisher's test was used. The constancy of variance across the four patient groups was checked using Bartlett's test. We used Classical Multidimensional Scaling based on Hamming distance to identify common treatment schemes. We examined infection risk and explanatory variables using logistic regression. Yates' correction for continuity was used when needed.

Ethical considerations

The research presented in this manuscript was approved by the Ethics Committee on Clinical Investigation of the Medical University of Warsaw.

Results

One hundred forty-three fecal samples collected from 58 IBD pediatric patients were screened for *C. difficile* toxin TcdA/TcdB, and toxigenic *C. difficile* bacteria. Twenty-five females and 33 males, aged between 3 and 18 years (mean age 11.9 years), participated in this study. Of all patients in the IBD group 21 had Crohn's disease (CD) and 37 had ulcerative colitis (UC). Baseline characteristics of pediatric patients and stool samples are shown in Table 1. All stool samples were negative for enteropathogenic organisms such as toxigenic *Escherichia coli*, *Salmonella* spp., *Shigella* spp., enterotoxigenic *C. perfringens*, and rota- and adenoviruses (data not shown). None of the patients required hospitalization for more than 3 months before fecal sample collection.

Table 1 Therapeutic treatment, demographic and number of patients and faecal samples from paediatric outpatients in active (A) and inactive (I) IBD

Demographic of patients and therapeutic treatment	CD[a] (n=21)		UC[a] (n=37)		Number of faecal samples from female and male and therapeutic treatment	Faecal samples (n=143) from IBD patients		CD[a] (n=49)		UC[a] (n=94)	
	A[b] (n=11)	I[b] (n=10)	A[b] (n=16)	I[b] (n=21)		A[c] (n=62)	I[c] (n=81)	A[c] (n=18)	I[c] (n=31)	A[c] (n=44)	I[c] (n=50)
Male (n=33)	6	8	10	9	Male (n=82)	36	46	11	25	25	21
Female (n=25)	5	2	6	12	Female (n=61)	26	35	7	6	19	29
Age 3-18 yr					Age 3-18 yr						
Sulfasalazine	3	0	4	16	Sulfasalazine	23	43	3	3	20	40
Mesalazine	6	7	13	6	Mesalazine	33	36	12	26	21	10
Azathioprine	8	7	10	10	Azathioprine	38	53	14	30	24	23
Cyclosporine	0	0	2	1	Cyclosporine	3	1	0	0	3	1
Steroids	6	3	13	6	Steroids	41	22	7	5	34	17
Infliximab	1	1	0	0	Infliximab	1	1	1	1	0	0

Abbreviations: [a] CD or UC - patients with Crohn's disease or ulcerative colitis, respectively

[b] number of patients (female or male) in active (A) or inactive (I) IBD.

[c] number of fecal samples in active (A) or inactive (I) IBD.

Table 2 Summary of detection of TcdA/TcdB toxins and/or toxigenic *C. difficile* strains in specimens of pediatric outpatients with IBD and recurrences of CDI

Number of faecal samples[a]	Number (%) of positive faecal samples for CDI[c]	Number of patients[d]	Number (%) of positive patients for CDI[c]	Number of patients with recurrences[e]	Distribution of common genotypes in patients with recurrences of CDI[f]
IBD[b] n=143	86/143 (60%)	IBD[b] n=58	40/58 (69%)	10/58 (17%)	014
n=62 (A)	42/86 (49%)	n=27 (A)	18 (66%)	5/27 (18%)	014
n=81 (I)	44/86 (51%)	n=31 (I)	22 (71%)	5/31 (16%)	014/010
CD[b] n=49	29/49 (59%)	CD[b] n=21	13 (62%)	3/21 (14%)	014
n=18 (A)	12/29 (41%)	n=11 (A)	7 (64%)	1/11 (9%)	014
n=31 (I)	17/29 (59%)	n=10 (I)	6 (60%)	2/10 (20%)	014/010
UC[b] n=94	57/94 (61%)	UC[b] n=37	27 (73%)	7/37 (20%)	014
n=44 (A)	30/57 (53%)	n=16 (A)	12 (75%)	4/16 (25%)	014
n=50 (I)	27/57 (47%)	n=21 (I)	15 (71%)	3/21 (14%)	014

Abbreviations: [a] number of fecal samples received from patients with IBD active (A) or inactive (I), [b] IBD -inflammatory bowel disease, CD-Crohn disease, UC-ulcerative colitis, [c] *C. difficile* toxins and/or toxigenic *C. difficile* strains, [d] -number of patients with IBD (CD or UC) active (A) or inactive (I), [f] PCR-ribotype of *C. difficile* strains; [e] number of patients with recurrences with IBD (A) or (I); [f] PCR-ribotypes caused recurrences in patients with IBD (A) or (I)

Clostridium difficile toxins were identified in 60% of the stool samples (86 out of 143) and in 69% of the pediatric patients (40 out of 58) participating in this study. The risk of *C. difficile* infection was independent of disease type (Crohn's disease or ulcerative colitis; $\chi^2=2.5821$, df=3, $p=0.4606$). TcdA/TcdB detection rates and *C. difficile* culture data are shown in Table 2. A highly significant relationship between the presence of *C. difficile* toxins and IBD, both active ($p<0.0001$) and inactive ($p<0.0001$), was observed. In addition, no significant correlation was found between *C. difficile* infection and IBD therapy, which included sulfasalazine ($p=0.1856$), mesalazine ($p=0.129$), azathioprine ($p=0.341$), and steroids ($p=0.8255$). Cyclosporine and infliximab were excluded from the statistical analysis because of their insignificant numbers.

From EIA-positive stool samples we cultured 18 *C. difficile* strains. PCR-ribotyping identified five different ribotypes: three among the toxigenic (014, 018, 046) and two among the non-toxigenic strains (010, 035). The most predominant ribotype was 014 ($A^+B^+CDT^-$) accounting for 50% of all strains. Two to seven clinical recurrences of *C. difficile* infection were observed during the study period among IBD patients and 17% of all 58 patients had recurrences (Table 2). Strains isolated during the first episode of *C. difficile* infection usually belonged to PCR-ribotype 014, and strains from the re-infections belonged to different ribotypes (ribotype 018 or 046). Colonization of the gastrointestinal tract with non-toxigenic *C. difficile* strains (ribotype 010 or 035) was very frequent among the IBD patients.

All *C. difficile* strains were susceptible to metronidazole (MIC range 0.023–0.38 mg/L), vancomycin (MIC range 0.25–1.5 mg/L), rifampin (MIC=0.002 mg/L for all strains), moxifloxacin (MIC range 0.064–1.5 mg/L) and gatifloxacin (MIC range 0.5–1.5 mg/L), but were resistant to ciprofloxacin (MIC≥32 mg/L).

Discussion

In our study, the prevalence of *C. difficile* infection in pediatric outpatients with IBD was assessed as being 69%. This is clearly higher than reported in the only previously published pediatric study (27.8%) [10] or among adults with IBD (4.2–5.8%) [6, 7]. Several factors could explain our results. First, the majority of our children were outpatients and previous studies showed that the majority of IBD patients contract *C. difficile* infections outside the hospital [6, 7]. A second possible reason is the way we controlled our patients: a fecal sample for toxin detection was collected during every follow-up visit, which are regularly arranged even in the absence of aggravation. Finally, the incidence of *C. difficile* infection in Polish pediatric IBD patients is unknown, but it could simply be higher than in Western Europe or the United States.

Large population-based studies have demonstrated that CDI is more common in ulcerative colitis (UC) than Crohn's disease (CD) [6, 7]. Our data showed that no specific type of IBD in our pediatric outpatients predisposed to *C. difficile* infection; the risk of *C. difficile* infection was the same in both UC and CD. This can be explained by the fact that the majority of our CD patients had colonic disease. IBD patients with colonic involvement exhibited a significant association with the development of *C. difficile* infection [16]. However, the lack of a comparator population (patients without IBD) limits the strength of the study.

We detected no significant correlation between the risk of *C. difficile* infection and IBD therapy, including anti-inflammatory drugs (sulfasalazine, mesalazine) or immune-modulating drugs (azathioprine, systemic steroids). These findings confirmed results from the published pediatric study [10], but not those from adult studies, which had shown that immune modulators were risk factors for *C. difficile* infection in hospitalized IBD patients [6, 17]. The impact of ongoing immune modulation on *C. difficile* infection in adult outpatients is as yet undefined.

Two, 3, 4, and 7 recurrences of *C. difficile* infection were observed during the study period in 1, 4, 4, and 1 patients respectively. Recurrences of *C. difficile* infection among IBD patients are a serious problem, especially for patients with 3 or more episodes [18]. Issa et al. demonstrated that 59% of the patients with IBD had a clinical recurrence within 1 month of the initial resolution of symptoms using metronidazole or vancomycin [19]. Another 22% had persistent symptoms with frequent relapses. The data regarding the recurrence rate of *C. difficile* in the adult IBD population are limited and there are no data at all for pediatric IBD patients [16]. Recurrences of *C. difficile* may be explained either by the endogenous persistence of a *C. difficile* strain (relapse) or by acquisition of a new strain from the environment (re-infection) [20]. We have shown both scenarios to be relevant. However, our data are limited because of small numbers of *C. difficile* isolates.

Two reports have previously shown that 38–56% of recurrent *C. difficile* infection were in fact due to re-infections [21, 22]. Barbut et al. showed that 48.4% of clinical recurrences among patients hospitalized in different clinical units in France were in fact re-infections with a different strain of *C. difficile* [20]. Our results point to frequent (re-)infection caused by one type of *C. difficile* (PCR-ribotype 014). Relapses followed each other more closely (less than 1 month between episodes) than re-infections. Metronidazole and oral vancomycin have been shown to be effective in the treatment of *C. difficile*-associated diseases, but symptomatic recurrences occur in 15–30% of cases [21–23].

Currently, there are no specific antibiotic regimens recommended for IBD patients suffering from CDI. Metronidazole is still considered the first-line therapy for *C. difficile* infection, so all of our patients were treated with metronidazole. In spite of reports that metronidazole treatment for *C. difficile* infection is effective in only 50% of patients [6], we did not observe resistance to metronidazole and vancomycin in pediatric *C. difficile* strains [24]. We observed that all strains isolated from pediatric IBD outpatients were susceptible to rifampin.

Fluoroquinolones are a mainstay of treatment in patients with IBD, particularly for management of suppurative complications such as abscesses in Crohn's disease [6]. Our findings confirmed that the resistance of *C. difficile* to ciprofloxacin was very frequent [9, 24, 25]. However, resistance to newer fluoroquinolones such as gatifloxacin and moxifloxacin was not observed among strains isolated from our patients with IBD.

Conclusions

We report a high prevalence of CDI in pediatric outpatients with inflammatory bowel disease, a clear risk of CDI in both ulcerative colitis and Crohn's disease. In addition, there was no significant correlation between *C. difficile* infection and IBD therapy, and PCR ribotyping revealed both *C. difficile* re-infection and relapses during episodes of IBD in pediatric outpatients.

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