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ORIGINAL ARTICLE

Clostridium difficile infection in children: epidemiology and risk of recurrence in a low-prevalence country

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Abstract *Clostridium difficile* infection (CDI) is increasingly found in children worldwide, but limited data are available from children living in southern Europe. A 6-year retrospective study was performed to investigate the epidemiology, clinical features, treatment, and risk of recurrence in Italy. Data of children with community- and hospital-acquired CDI (CA-CDI and HA-CDI, respectively) seen at seven pediatric referral centers in Italy were recorded retrospectively. Annual infection rates/10,000 hospital admissions were calculated. Logistic regression was used to investigate risk factors for recurrence. A total of 177 CDI episodes was reported in 148 children (83 males, median age 55.3 months), with a cumulative infection rate of 2.25/10,000 admissions, with no significant variability over time. The majority of children (60.8 %) had CA-CDI. Children with HA-CDI (39.2 %) had a longer duration of symptoms and hospitalization ($p = 0.003$) and a more common previous use of antibiotics ($p = 0.0001$). Metronidazole was used in 70.7 % of cases (87/123) and vancomycin in 29.3 % (36/123), with similar success rates. Recurrence occurred in 16 children (10.8 %), and 3 (2 %) of them presented a further treatment failure. The use of metronidazole was associated with a 5-fold increase in the risk of recurrence [odds ratio (OR) 5.18, 95 % confidence interval (CI) 1.1–23.8, $p = 0.03$]. Short bowel syndrome was the only underlying condition associated with treatment failure (OR 5.29, 95 % CI 1.17–23.8, $p = 0.03$). The incidence of pediatric CDI in Italy is low and substantially stable. In this setting, there is a limited risk of recurrence, which mainly concerns children treated with oral metronidazole and those with short bowel syndrome.

Abbreviations

CD	*Clostridium difficile*
CDI	*Clostridium difficile* infection
HA-CDI	Hospital-acquired CDI
CA-CDI	Community-acquired CDI
PCR	Polymerase chain reaction
EIA	Enzyme immunoassay
GDH	Glutamase dehydrogenase
SITIP	Italian Society of Pediatric Infectious Diseases

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Introduction

A dramatic increase in the incidence of *Clostridium difficile* infection (CDI) has been reported in the past decade worldwide. This change in epidemiology is probably linked to an excess of antibiotics and other causes of microflora disruption, to the concomitant emergence of hypervirulent epidemic strains (e.g., NAP1/BI/027), and to the increase of potentially highly susceptible individuals [1–3]. The role of *C. difficile* (CD) in infancy and

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childhood is still a matter of debate. This sporogenic anaerobic organism is responsible for a broad spectrum of diseases in children, ranging from a self-limiting secretory diarrhea to life-threatening conditions, such as pseudomembranous colitis, toxic megacolon, intestinal perforation, and septic shock [3]. On the other hand, asymptomatic CD colonization is common in infants and young children (2–75 %), and seems to be related to factors such as age, type of delivery, and feeding [4]. However, the recent change in epidemiology has been confirmed also for the pediatric population, and some evidence reports a significant increase of both hospital- and community-acquired CDI (HA-CDI and CA-CDI, respectively) incidence in children living in western countries [5, 6], with rates that vary between 12.8 and 40/10,000 hospital admissions, according to different populations [7, 8].

Recurrence is a relatively common complication of CDI and about 20–30 % of children with CDI experience a new episode of infection after first-line treatment [5, 9, 10]. Risk factors for recurrent CDI in children have been recently reported and include previous use of antibiotics, malignancy, recent surgery, and presence of respiratory devices [9–11].

The epidemiology and risk factors of CDI in children living in southern Europe and Mediterranean countries are poorly known. Available evidence is limited to small populations (less than 30 cases) or sporadic case reports [12–14].

The Italian Society of Pediatric Infectious Diseases (SITIP) promoted a retrospective study to investigate the epidemiology of CDI in Italian children and analyze clinical features, therapeutic options, outcomes, and risk factors for recurrence.

Materials and methods

We conducted a 6-year retrospective, multicenter, observational study including all children (birth to 18 years of age) with confirmed CDI admitted between January 2008 and December 2013 in seven large pediatric centers in Italy. The enrolling centers provide tertiary care to Italian children. Most children seen in these institutions belong to at-risk populations with chronic diseases.

Definitions

CDI was defined by the presence of diarrhea, defined as ≥3 watery or loose stools in the previous 24 h [15], associated with a positive finding of toxigenic CD in the stools (toxins A and/or B or binary toxin) or the presence of pseudomembranous colitis on endoscopy or histology, according to recent guidelines [16, 17]. The modality of CD detection slightly varied according to institution and year of enrollment. Three institutions used enzyme immunoassay (EIA) for the whole study period. Real-time polymerase chain reaction

(rPCR) for CD toxins was used in one institution for the whole study period. Another institution used EIA from 2008 to 2010 and then introduced rPCR for CD toxins. Two institutions used a two-step diagnostic approach with glutamase dehydrogenase (GDH) test as the first step, and successive confirmation of the strain toxigenicity rPCR for the toxins. CD culture was not performed routinely.

The presence of fever and vomiting, and the main characteristics of stools (including mucus and/or blood) were recorded, together with other clinical data. CD-positive children were included in the study only if other routine microbiological investigations for common intestinal pathogens were negative (i.e., rotavirus and standard stool culture for *Salmonella*, *Shigella*, or *Campylobacter*).

According to guidelines, recurrent CDI was defined if diagnostic criteria of CDI were met within 8 weeks of initial diagnosis after documented symptom resolution [16, 18, 19]. Infection was defined as hospital-acquired (HA-CDI) if onset of symptoms occurred >48 h after admission, or less than 4 weeks after discharge from a healthcare facility. Infection was defined as community-acquired (CA-CDI) if onset of symptoms occurred in the community or within 48 h of admission to a hospital, or if symptom onset was >12 weeks after the last discharge from a hospital. Infection was defined as indeterminate if symptom onset occurred between 4 and 12 weeks from a hospital discharge; however, indeterminate CDI in our population ($n = 1$) was classified as community-acquired for study purposes [17].

Data collection

The pediatricians managing single patients, in collaboration with the microbiology units of each center, collected the following data retrospectively by using standardized forms: patient's age, gender, underlying conditions, or prematurity; date and ward at hospital admission, CDI-related signs and symptoms, recent healthcare-associated exposures including administration of antibiotics in the past 2 months, use of immunosuppressive therapy, CDI treatment including antibiotics (drug, formulation, and dose), probiotics (strain) and other treatments, patient outcomes (including treatment failure, recurrence, and death), duration of symptoms, and length of hospital stay and treatment. All clinical and demographic data were extracted manually from medical records and the forms were sent to the coordinator center and inserted into a Microsoft Office Excel workbook.

In order to calculate the annual infection rate, each center reported to the coordinator the total number of hospital admissions for each year of observation.

The study was approved by the Council of the SITIP (session of May 2014) and has been conducted according to the principles expressed in the Declaration of Helsinki. All patients' data were analyzed and reported anonymously.

Statistical analysis

Infection rates were reported as the number of CDIs recorded in single centers normalized for 10,000 hospital admissions for each year of observation. The number of admissions was limited to pediatric patients for those centers that also mange adults. Cumulative infection rates with relative 95 % confidence intervals (CIs) for annual infection rates were used to compare infection rates according to the year of observation and to the diagnostic method for CD detection.

Continuous variables were summarized and reported as means and standard deviation (SD). Categorical variables were summarized and reported as frequencies and percentages. Clinical characteristics and underlying conditions of children with HA-CDI and CA-CDI were compared by using the t-test and non-parametric the Mann–Whitney test. The age of children was reported in months and interquartile range (IQR). Categorical variables were compared by means of Fisher's exact test or the Chi-square test, as appropriate. Univariate analysis of variables influencing the risk of recurrence was performed using the logistic regression analysis and Cox's regression model. Risk was reported as odds ratio (OR) with 95 % CI. Multivariate analysis considered all therapeutic options included in the univariate analysis and the setting of CDI. Two-sided p-values ≤0.05 were considered statistically significant. Statistical analysis was performed using the SPSS Statistics software (version 20.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

CDI rates and clinical features

In the 6-year study period, 167 CDI episodes were recorded in 148 children (83 males, median age 55.3 months), with a cumulative infection rate of 2.25/10,000 hospitalized children. The general characteristics, clinical features, and underlying clinical conditions of enrolled children are reported in Table 1. The infection rate varied from a maximum of 5.1 in 2009 to a minimum of 1.15/10,000 in 2010 (Fig. 1). The infection rates with 95 % CI did not change during the study period ($p > 0.05$). No significant difference was observed among different institutions, although a slight (but not significant) trend toward increase was observed in centers located in northern Italy when compared to those in the southern part of the country. This result was confirmed also when the infection rates were analyzed according to the diagnostic methods used for CD detection in different enrolling centers (Table 2). Although the centers using molecular diagnostic tests (mainly the two-step diagnostic approach with GDH and rPCR) showed slightly higher rates than the others, no significant difference in

infections rates (with 95 % CI) was observed in the 6 years of observation (Table 2).

The majority of infections (60.8 %) met the definition of CA-CDI (Table 1). Children who experienced HA-CDI (58, 39.2 %) had a longer hospitalization ($p = 0.003$) and a slightly longer duration of symptoms (Table 1).

The majority of children (82.5 %) had underlying conditions that can increase the risk of CDI; however, this pattern was more frequent in HA-CDI than in CA-CDI (Table 1). Previous use of antibiotics was reported in 79 patients (53.4 %), being significantly more common in children with HA-CDI ($p = 0.0001$) (Table 1).

Chronic bowel diseases (50, 33.7 %) and hemato-oncologic diseases (27, 18.2 %) were the most commonly reported underlying chronic conditions. Chronic renal diseases and history of prematurity were significantly more common in children presenting with HA-CDI. Inflammatory bowel diseases were more common in children with CA-CDI (Table 1).

A child with leukemia and HA-CDI died after the first treatment with vancomycin and probiotics, resulting in a mortality rate of 0.67 %.

CDI treatment

Of the 148 patients, 123 underwent specific antibiotic treatment (83.1 %); in the remaining patients, symptoms resolved rapidly and without treatment. The first-line treatment was metronidazole in 70.7 % of children (87/123) and vancomycin in 29.3 % (36/123). Among children with HA-CDI, metronidazole was prescribed in 63.8 % of cases and vancomycin in 24.1 %. A similar proportion was observed in CA-CDI, 55.6 % and 24.4 %, respectively. Although the rate of not treated children was slightly higher in CA-CDI (20 %) than HA-CDI (12 %), this difference was not statistically significant ($p = 0.39$). Among the 25 patients (16.9 %) who did not receive specific antibiotic treatment for CD, 15 (60 %) had a recent exposure to antibiotics (mainly penicillins, cephalosporins, and fluoroquinolones), three had inflammatory bowel diseases, one had a history of minimal bowel resection, and the remaining six had no underlying conditions. Most of these patients received diagnosis by using rPCR.

Probiotics were used in 35 episodes of CDI. In most cases (28/35, 80 %) they were used in association with antibiotics for the treatment of HA-CDI (15, 53.5 %) or CA-CDI (13, 46.4 %). However, in 7 episodes (20 %), probiotics were used as the only treatment. Nine different formulations were used (single strains or mix of probiotics); *Lactobacillus reuteri* was the most common single strain reported (10/35, 28.5 %).

No difference in treatment success rate was observed comparing children receiving probiotics or not, and the same results were observed if outcomes were analyzed according to different probiotic strains.

Table 1 Clinical features of 148 Italian children with community-acquired and hospital-acquired *Clostridium difficile* infections*

Parameters	All CDI	CA-CDI	HA-CDI	*p*-Value
No. of children (%)	148	90 (60.8)	58 (39.2)	
Male (%)	83 (56.1)	52 (57.7)	31 (53.4)	0.60
Median age (months, IQR)	55.3 (87.7)	47.3 (79.2)	59.7 (90.8)	0.28
Symptoms				
Duration of symptoms (mean days ± SD)	18.56 (27)	15.3 (23.1)	23.4 (31.7)	0.07
Duration of hospitalization (mean days ± SD)	25.1 (46.3)	13.9 (27.2)	42.5 (62.2)	0.003
Fever > 8 °C	62 (41.8)	34 (37.7)	28 (48.2)	0.23
Bloody stools	40 (27.0)	24 (26.6)	16 (27.5)	1.00
Vomiting	38 (25.6)	25 (27.7)	13 (22.4)	0.56
Mucus in stools	27 (18.2)	19 (21.1)	8 (13.7)	0.28
Moderate-to-severe dehydration	23 (15.5)	14 (15.5)	9 (15.5)	1.00
Abdominal pain	12 (8.1)	9 (10)	3 (5.1)	0.36
Ileus	4 (2.7)	1 (1.1)	3 (5.1)	0.30
Underlying conditions				
Previous use of any antibiotics (%)	79 (53.4)	38 (42.0)	42 (72.4)	0.0001
Previous use of ≥2 antibiotics (%)	37 (25)	13 (14.4)	24 (41.4)	0.0001
Inflammatory bowel diseases (%)	23 (15.5)	19 (21.1)	4 (6.8)	0.021
Non-inflammatory intestinal diseases (%)	14 (9.5)	9 (10)	5 (8.6)	0.95
Short bowel (%)	13 (8.7)	8 (8.8)	5 (8.6)	0.99
Oncologic diseases (%)	19 (12.8)	10 (11.1)	9 (15.5)	0.46
Chronic neurologic diseases (%)	11 (7.4)	4 (4.4)	7 (12)	0.11
Chronic renal diseases (%)	8 (5.4)	1 (1.1)	7 (12)	0.006
Bone marrow transplantation (%)	6 (4.0)	2 (2.2)	4 (6.8)	0.21
History of prematurity (%)	4 (2.7)	0 (0)	4 (6.8)	0.022
Solid organ transplantation (%)	2 (1.3)	1 (1.1)	1 (1.7)	0.99
Immunodeficiency and HIV infection (%)	1 (0.6)	1 (1.1)	0 (0)	1.00
No at-risk conditions (%)	26 (17.5)	22 (24.4)	4 (6.8)	0.007

CDI: *Clostridium difficile* infection; CA-CDI: community-acquired CDI; HA-CDI: hospital acquired-CDI; HIV: human immunodeficiency virus

*Data reported in the table refer to the first episode of infection

Fig. 1 *Clostridium difficile* infection (CDI) rates among children living in Italy. The *bars* report the rates for each year of observation from 2008 to 2013 normalized for 10,000 hospital admissions. The *dotted line* indicates the cumulative rate, calculated by dividing the total number of episodes of CDI by the total number of hospital admissions normalized for 10,000 admissions. The *p*-values express the significance of variation among all different annual rates; values <0.05 are considered to express significant variation. NS: not significant
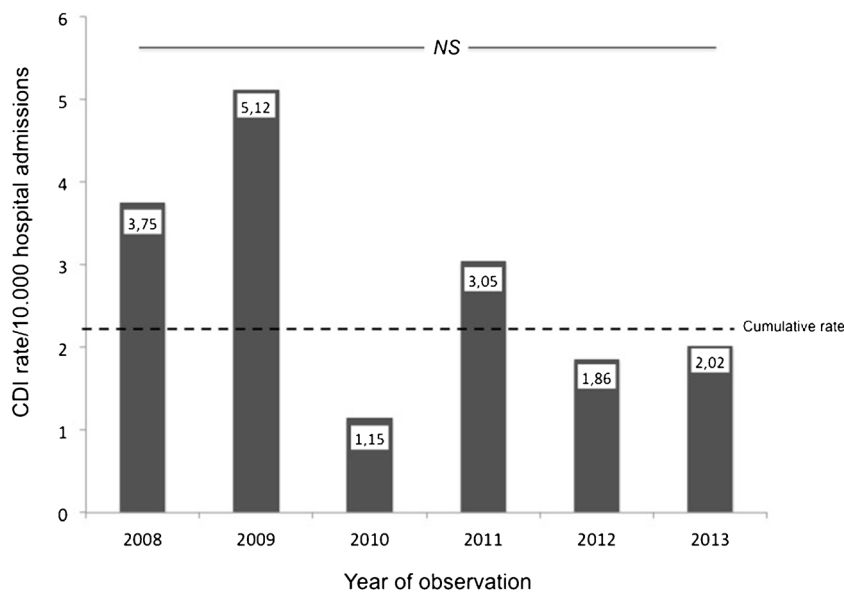
Table 2 Infection rates according to diagnostic method for the detection of *Clostridium difficile*

Diagnostic method	*Clostridium difficile* infection rate (95 % CI)						*p*-Value
	Year of observation						
	2008	2009	2010	2011	2012	2013	
EIA	5.85 (2.62–13.0)	1.94 (0.73–5.17)	6.38* (2.65–15.3)	1.26 (0.17–8.94)	1.22 (0.17–8.66)	2.45 (0.61–9.79)	NS
GDH/PCR	2.63 (1.09–6.31)	6.41 (3.55–11.6)	3.55* (1.59–7.90)	7.29* (4.03–13.1)	2.84 (1.06–7.56)	5.32 (2.53–11.1)	NS
PCR	–	–	0.45 (0.18–1.2)	2.6 (1.8–3.74)	1.78 (1.14–2.75)	1.58 (0.98–2.54)	NS

EIA: enzyme immunoassay for *Clostridium difficile* toxins A and/or B; PCR: polymerase chain reaction for *Clostridium difficile* toxins A and/or B; GDH/PCR: two-step methodology: glutamase dehydrogenase (GDH) test as the first step, and successive confirmation of the strain toxigenicity Real-time polymerase chain reaction (rPCR) for the toxins; NS: Not significant

p-Values express the difference among the CDI rates and is calculated comparing the 95 % CI of single annual rates; values <0.05 are considered significant

*$p < 0.05$ if compared to PCR

CDI recurrence

First-line treatment was successful in 89.2 % of children (133/148). Sixteen children (10.8 %) experienced a recurrence and 3 (2 %) of them presented a further failure after the second treatment. Recurrences were treated with metronidazole in 10 cases (62.5 %) (oral in seven cases and intravenous in three) and vancomycin in 6 cases (37.5 %). The two antibiotics had similar success rates in resolving CDI symptoms (80 % and 83.3 %, respectively, $p = 1.00$).

Table 3 reports the analysis of potential risk factors for CDI recurrence. Children with short bowel syndrome had a higher risk of developing CDI recurrence (OR 5.29, 95 % CI 1.17–23.8, $p = 0.03$). The risk of recurrence was similar between children with HA-CDI and CA-CDI. None of the other well-known risk factors were associated to higher risk of recurrence.

The use of metronidazole was associated with a 5-fold increase in recurrence, and this risk was detected in both the univariate and the multivariate analyses (Table 4). When differentiated according to the route of administration, this risk was confirmed for oral metronidazole (OR 3.24, 95 % CI 1.00–10.7, $p < 0.05$), but not for the intravenous route (OR 1.42, 95 % CI 0.28–6.91, $p = 0.66$). The dose of metronidazole used ranged between 15 and 40 mg/kg/day; however, doses <30 mg/kg/day were not significantly related to the risk of recurrence ($p = 0.26$). The association of probiotics with metronidazole reduces the risk of first-line treatment failure (Table 4). In addition, three recurrences were treated with this association and none of them experienced further episodes.

Table 3 Risk factors for *Clostridium difficile* infection recurrence in 148 Italian children

Risk factors	OR	95 % CI	*p*-Value
Hospital- vs. community-acquired CDI	1.89	0.65–5.5	0.24
Any underlying chronic disease	3.24	0.40–25.8	0.26
Inflammatory bowel diseases	0.96	0.19–4.7	0.96
Other chronic bowel diseases	2.75	0.65–11.53	0.16
Short bowel syndrome	5.29	1.17–23.8	0.03
Chronic neurologic diseases	0.87	0.10–7.38	0.90
Chronic renal diseases	0.94	NA	0.33
History of prematurity	3.09	0.30–31.7	0.34
Hemato-oncologic diseases	1.82	0.46–7.1	0.38
Bone marrow transplantation	1.82	0.19–16.7	0.59
Solid organ transplantation	0.2	NA	0.63
Use of immunosuppressive drugs	1.2	NA	0.27
HIV infection	0.1	NA	0.73
Co-infection with enteric pathogens	0.99	0.98–1.00	0.47

OR: odds ratio; CI: confidence interval; NA: not assessable

Discussion

The scientific and medical interest about CDI increased worldwide in the last decade following the evidence of significant changes in epidemiology and increasing morbidity reported in the adult population. Although the problem among children seems smaller, the pediatric world is moving in the same direction, and an increase in CDI incidence has been reported in North America [5, 7, 8] and northern Europe [20].

We found a CDI rate that was substantially stable in the 6-year period of observation, with an infection rate that varies from 3.75 in 2008 to 2.02/10,000 hospital admissions in 2013.

These findings contrast with a previous Italian experience that reported a trend toward increase in the last several years [14]. However, in that study, the authors reported results from a smaller population, seen at a single center and diagnosed by using a two-step diagnostic protocol, based on the GDH test and rPCR for toxins. In our experience, the centers using this two-step diagnostic approach showed higher infection rates

Table 4 Impact of treatment on the risk of *Clostridium difficile* infection recurrence

Treatment	Univariate analysis			Multivariate analysis		
Any recurrence	OR	95 % CI	*p*-Value	OR	95 % CI	*p*-Value
Metronidazole alone	5.18	1.1–23.8	0.03	6.38	1.01–40.1	0.04
Vancomycin alone	0.44	0.09–2.0	0.30	1.42	0.21–9.4	0.71
Probiotics alone	1.28	0.14–11.2	0.82	1.68	0.47–5.9	0.41
Metronidazole + probiotics*	2.52	0.62–10.1	0.19	–	–	–
Vancomycin + probiotics*	0.82	NA	0.36	–	–	–
Two or more recurrences	OR	95 % CI	*p*-Value	OR	95 % CI	*p*-Value
Metronidazole alone	0.69	0.95–5.06	0.71	1.8	0.13–24.1	0.65
Vancomycin alone	3.21	0.43–23.8	0.24	4.8	0.36–65.3	0.23
Metronidazole + probiotics	4.11	0.35–30.7	0.16	4.39	0.58–35.3	0.51

OR: odds ratio; CI: confidence interval; NA: not assessable

*Single daily dose of different probiotic strains added to antibiotic treatment, including: *Lactobacillus reuteri* (*n* = 10), *Lactobacillus rhamnosus* GG (*n* = 2), *Lactobacillus casei* DG (*n* = 1), *Lactobacillus rhamnosus* (*n* = 1)

(mean rate 4.67/10,000) than those recorded in centers using EIA or rPCR alone (3.18/10,000, $p > 0.05$).

In addition, it should be noted that CDI rates recorded in Italy are 5- to 10-fold lower than those reported in children living in the United States, where the rates vary between 12.8 and 40/10,000 admissions [7, 8]. This difference is relevant even if the incidence is calculated only in Italian centers using molecular diagnosis (below 5/10,000, Table 2).

These findings demonstrated that the epidemiology of pediatric CDI in a southern European country, such as Italy, might be significantly different from northern Europe and America. The reasons for this difference are unclear and need to be specifically investigated; however, factors such as lifestyle or diet, which dramatically affect the composition of intestinal microbiota, may play a relevant role. A further hypothesis that may explain a variation in epidemiology is the potential exposition to contaminated foods, whose distribution may vary from country to country. Toxigenic CD strains have been found in ready-to-eat salad, meat, shellfish, dairy products, and wastewater [21–24], and the use of these products may account for part of community-acquired CDI. The latter is responsible for the majority of cases in pediatric reports [5, 25], as well as in our population.

On the other hand, the evidence of a low CD prevalence in Italy is also surprising, considering that CDI is strongly related to the use and abuse of antibiotics [26, 27], and that the consumption of antibiotics in children living in Italy is significantly higher than that reported in other European countries [28–30]. In contrast, the use of antibiotics is strictly under control in poultry and foodstuffs.

Current antibiotic treatments for CDI are able to resolve 90 % of acute CDI; however, treatment failure is certainly the major problem in the management of patients with CDI in either adulthood or childhood. In addition, relapses/

recurrences are associated with increased severity and poor outcomes. Several new therapeutic approaches have been proposed in the last several years to reduce the rate of recurrence. Although selected treatment options such as fidaxomicin [31], fecal microbiota transplantation [32–34], or CD toxin-targeted monoclonal antibodies [35, 36] are gaining importance and consensus in adult medicine, most of them have not been tested in large pediatric populations [37].

In our study, 10 % of children experienced a recurrence after first-line treatment. This rate is significantly lower than that reported in North America, where 18–31 % of children present a recurrence [5, 38].

We found that children receiving metronidazole alone as first-line treatment had a 5-fold higher risk of developing recurrence compared to children receiving vancomycin or a combination of antibiotics and probiotics. The risk seems to be related to oral administration rather than the intravenous route; however, this difference should be considered with caution, since only a small proportion (about 5 %) of children received intravenous doses. Metronidazole is almost completely absorbed in the small intestine; however, its concentration in the colon varies according to stool pattern and intestinal motility [39]. In any case, several factors might influence the efficacy of oral administration in children, including the difficulty of administration in younger children due to the lack of pediatric formulation, the drug dilution entrusted to families, the co-administration of other drugs, and the variability in drug doses. In our population, oral metronidazole was administered in variable doses ranging from 15 to 40 mg/kg/day; however, the change in dose was not related to the risk of CDI recurrence.

Although we found wide 95 % CIs, this finding was confirmed in both univariate and multivariate analyses. The width of intervals reported may be related to the relatively small size

of the population and to the difference in the number of patients receiving metronidazole and vancomycin as first-line treatment.

Evidence in adults demonstrated no difference in recurrence between metronidazole and vancomycin for both mild and severe CDI, neither in monotherapy nor in combination [40, 41]. However, although evidence from pediatric age patients is more limited, Khanna et al. reported an 18 % recurrence rate in children receiving metronidazole as first-line treatment and no recurrence in those receiving vancomycin [5], in keeping with our results.

The CD resistance to metronidazole might partially justify the relapse after first-line treatment; however, this is a rare event reported in 1–6.5 % of pediatric cases [42]. In our experience, CD culture was not routinely performed for the diagnosis of CDI. For that reason, and for the retrospective nature of the study, data on CD antibiotic resistance profile was available only for a minority of patients enrolled in a single center. In the 18 specimens available for culture (12 % of total events), no in vitro resistance to metronidazole was observed.

Position papers released by relevant societies currently recommend metronidazole as first-line treatment for the initial episode of CDI in children [43, 44]. This recommendation is based on weak evidence, but is essentially aimed to minimize the overuse of vancomycin and reduce the potential risk of emergence of antibiotic resistance. Any potential difference in the risk of CDI recurrence between metronidazole and vancomycin needs to be specifically investigated in prospective studies on pediatric population. However, whether our findings would be confirmed by further data, the role of oral metronidazole as first-line treatment would need to be reviewed.

Some underlying conditions have been recently identified as risk factors for recurrent CDI in children, including previous use of antibiotics, malignancy, recent surgery, and tracheotomy [9–11]. In our population, short bowel syndrome was the only underlying condition that significantly predisposed to CDI recurrence. Patients with this condition underwent major intestinal surgery, received parenteral nutrition, and, not rarely, undergo heavy antibiotic therapy for central-line infections, intestinal bacterial overgrowth, and other infections. All these factors might play a role in the proliferation of CD, disruption of intestinal microflora, and the potential development of CDI recurrences.

The retrospective nature represents a limitation of our study. In order to study the epidemiology of an emerging infection like CDI, prospective data on large populations are needed. However, this retrospective study collected data from several representative pediatric tertiary care centers in Italy, covering a quite extended period and provided information on the largest pediatric population currently published in European countries. A further limitation of our study is the variability in CD detection methodology that was recorded in the enrolling institutions during the study period. New, more sensitive diagnostic approaches, including toxin-targeted rPCR or a two-step approach with GDH and rPCR have been proposed and largely introduced in many institutions. These diagnostic assays are changing the capability of detecting CD in stools [45], but also have a relevant impact on the epidemiology of CDI [46]. In our observational study, these methods were used during the study period by three institutions and one institution introduced rPCR in 2010. However, to minimize this bias, infection rates were analyzed in accordance with the diagnostic assay and no significant difference in incidence was observed during the study period.

In conclusion, we demonstrated that CDI has a low and stable incidence and a limited morbidity in children in Italy. The infection rate and risk of recurrence are significantly lower than those reported in other western countries. In this context, children receiving oral metronidazole as first-line treatment and those with short bowel syndrome are at risk of developing CDI recurrence.

Further studies on large pediatric populations are needed to monitor infection in other European countries and investigate potential factors that can impact on the risk of CDI in this population.

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