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REVIEW

Co-infection as a confounder for the role of *Clostridium difficile* infection in children with diarrhoea: a summary of the literature

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Abstract Although *Clostridium difficile* is a major cause of antibiotic-associated diarrhoea in adults, the incidence and severity of *C. difficile* infection (CDI) in children is unclear. One complicating factor in assessing the role of CDI in children is the possibility of co-infection with other gastrointestinal pathogens. In this review, we summarise the literature concerning *C. difficile* co-infections in young children, in an attempt to discuss the rate of co-infections and their potential role in the severity of CDI clinical presentation. We identified 31 studies where co-infections were analysed, comprising 1, 718 patients with positive *C. difficile* tests. The pooled percentage of reported co-infections was 20.7 % (range 0–100 %). Viral co-infections were most commonly reported (46 %), with bacteria and parasites accounting for 14.9 % and 0.01 % of cases, respectively. However, the panel of co-infections tested for varied considerably among studies and 38 % of stated co-infections did not have a pathogen reported. Substantial variation in how and when tests for gastrointestinal co-infections are carried out, small sample sizes and a lack of clear CDI case definitions preclude meaningful conclusions on the true rate of co-infections in this patient population. This review suggests that co-infections may be common in children with diarrhoea who tested positive for *C. difficile*. Given a lack of CDI case definitions, especially in young children under the age of 5 years, a broad panel of pathogens should be tested for to exclude other microbiological causes. However, the summarised poor quality of the available literature on this subject highlights a need for further studies.

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Introduction

Clostridium difficile is a Gram-positive, anaerobic, spore-forming bacillus that capitalises on disruption of the normal intestinal microbiota to colonise the large intestine, causing disease symptoms through the action of its toxins [1–3]. *C. difficile* infection (CDI) is associated with significant morbidity and mortality in adults [2], but the incidence and severity of CDI in neonates and infants is currently unclear. Diarrhoeal illness is very common in young children in whom high carriage rates of *C. difficile* are reported [4]. Alongside a lack of clear CDI case definitions in those children under the age of 3 years [5], detection of *C. difficile* is commonly interpreted as asymptomatic colonisation and not the causative agent for diarrhoea. However, there have been reports of a potential pathogenic role of *C. difficile* in this patient population, as occurs in adults [4, 6, 7]. A recent study suggested that the use of adult markers of disease severity are not useful in guiding the management of CDI in children ≤16 years of age, which makes it difficult to design and interpret clinical studies [8].

A key complicating factor in assessing the pathophysiology of *C. difficile* in children is that detection of *C. difficile* in children with diarrhoea can be indicative of colonisation only, and co-infection with another gastrointestinal pathogen can be the true cause of the disease. Studies have shown that rates of positive *C. difficile* tests are similar in stools of asymptomatic

young children and children with diarrhoea [9, 10]. Consequently, it has been suggested that, if *C. difficile* is detected in children under 3 years of age, alternative causative agents for diarrhoea should be sought [11, 12]. Currently, the precise rate of co-infections in *C. difficile*-positive children, and the role co-infections play in disease severity, is not known. In this article, we review the literature in an attempt to discuss the prevalence of co-infection with *C. difficile* and other pathogens in children under 18 years of age with diarrhoea, the effect of co-infections on CDI severity and variations in diagnostic testing practises.

Literature search criteria

PubMed and EMBASE were searched for all citations with *C. difficile* and children using the search string (*Clostridium difficile* OR *C. difficile* OR *difficile*) AND (child OR child* OR infan* OR neonat* OR baby OR babies OR pediatric OR paediatric OR adolescen*). The search was limited to articles, and articles cited therein, published from January 1st 1980 until the date of search on December 13th 2013. Studies were excluded if they were not published in English, German, French or Dutch; if no investigations for co-infection were performed; if the study did not contain original data; if the paediatric population could not be separated from adult patients; or if the study population was smaller than 50 patients (to reduce the potential bias induced when taking into account case reports or case series).

Studies included in the analysis

A total of 1,333 hits were obtained in the literature search, which were then screened based on title and abstract. Articles were excluded for the following reasons: not in English/German/French/Dutch (n=131), no mention of co-infection (n=603), review without original data (n=161), the paediatric population could not be separated from the adult patients (n=135) and study population of <50 patients (n=221). The full texts of the remaining 82 articles were screened and 51 were excluded because no data were available on co-infections. Thirty-one studies were included, incorporating a total of 10,201 patients meeting individual study inclusion criteria, of which 1,718 patients had a positive *C. difficile* test and diarrhoea (Table 1). Studies included children aged 0–2 years (n=4), 0–5 years (n=2), 0–12 years (n=3) and 0–18 years (n=22). The majority of studies were from North America (n=12; 6,184 cases) [13–24] and Europe (n=12; 2,467 cases) [8, 25–35], with other studies from Asia (n=4; 1,762 cases) [36–39], Australia (n=2; 148 cases) [40, 41] and South America (n=1; 210 cases) [42]. Ten studies included only community-onset patients [13, 14, 25, 27, 28, 31, 32, 34, 35, 38], three included only hospital patients [20, 30, 40], 12 included both hospital and community [8, 15, 16, 19, 21–24, 26, 29, 33, 42] and six studies did not report the place of onset [17, 18, 36, 37, 39, 41]. Co-morbidities cited in the studies included cancer, transplantation, immunosuppression, inflammatory bowel disease and bone marrow transplantation. No studies described the inclusion of patients during an outbreak of gastrointestinal disease.

Rate of *C. difficile* co-infection with other gastrointestinal pathogens

Of the 10,201 patients included in all the studies, a total of 1,708 (16.1 %) *C. difficile*-positive tests were obtained from patients with diarrhoea, using a variety of diagnostic methodologies, which are summarised in Table 1. In this group, a total of 355 co-infections were reported (pooled percentage 20.8 %). Reported co-infection rates varied between 0 and 100 %, with seven studies reporting a co-infection rate of ≥50 % among *C. difficile*-positive patients (Fig. 1).

The frequencies of co-infection in each study are described in Table 1. We found that the panel of co-infecting pathogens tested for varied substantially among the 31 included studies. Only four systematically tested for viruses, bacteria and parasites in all cases [23, 30, 33, 37]. The study by Oğuz et al. of 100 children (aged 0–13 years) with diarrhoea isolated a co-infecting pathogen in 25 % (6/24) of those with a positive *C. difficile* test, of which rotavirus was isolated in four cases and *Entamoeba histolytica* in two [30]. Shastri et al. screened stool samples from 267 children (aged 0–16 years) based on symptoms of vomiting, diarrhoea or feeding intolerance, and identified an astrovirus co-infection in 10 % (4/40) of those with a positive *C. difficile* test [23]. The study by Uhnoo et al. analysed stool samples from 616 children (aged 0–14 years) and identified a co-infecting pathogen in 45.3 % (39/86) of *C. difficile*-positive patients (rotavirus, n=19; adenovirus, n=12; calicivirus, n=1; *Y. enterocolitica*, n=2; *C. jejuni*, n=3; enteropathogenic *E. coli*, n=1; *S. typhimurium* + *C. jejuni* + *E. coli*, n=1) [33]. Albert et al. analysed stool samples from 814 children with diarrhoea (aged 0–5 years), noting a co-infection in 53.8 % (7/13) of those with a positive *C. difficile* test (rotavirus, n=2; *C. jejuni*, n=1, enteropathogenic *E. coli*, n=1, enterotoxigenic *E. coli*, n=1, *Aeromonas* spp., n=1; *Shigella* spp., n=1) [37]. Twenty studies tested for bacterial co-infection in all samples [13, 18, 19, 23, 24, 27–41], 13 tested for viral pathogens (of which five tested for rotavirus only) [16, 18, 19, 23, 27–31, 33–35, 37] and six tested for parasites [23, 30, 32, 33, 37, 40]. In ten studies, not all samples were tested for co-infection or no data were reported on the number of tested samples [8, 14, 15, 17, 20–22, 25, 26, 42].

The number of reported co-infections by pathogen is described in Table 2. We also observed that, where co-infections

Table 1 Studies included in the analysis and the reported rate of *Clostridium difficile* co-infection with other gastrointestinal pathogens

Study	No. of patients	Patient age range	No. of samples tested for CDI, *n* (%)	CDI testing method	CDI +ve test with diarrhoea, *n* (%)	Rate of co-infection among *C. difficile*-positive samples, *n* (%)
Ahmad et al., 1993 [36]	100	<12 years	100 (100)	Culture+cytotoxicity	18 (18.0)	4 (22.2)
Albert et al., 1999 [37]	814	<5 years	814 (100)	Cytotoxicity	13 (1.6)	7 (53.8)
Bauer et al., 2009 [25]	148	<19 years	148 (100)	Toxin immunoassay	6 (4.1)	0 (0)
Boenning et al., 1982 [13]	306	2 weeks–16 years	306 (100)	Culture	12 (3.9)	1 (8.3)
Burgner et al., 1997 [40]	60	Mean 5 years 6 months	149 (100)	Culture	13 (8.7)	2 (15.4)
Denno et al., 2005 [14]	104	<21 years	104 (46.0)	Cytotoxicity	8 (7.7)	1 (12.5)
Deorari et al., 1999 [15]	173	0.13–215 months	173 (33.1)	Culture + toxin immunoassay	46 (26.6)	2 (4.3)
Dulęba et al., 2014 [26]	60	3 months–16 years	Inclusion criteria	Toxin B/CDT PCR	64 (100)	12 (18.8)
El Feghaly et al., 2013 [16]	62	2.3–15.7 years	Inclusion criteria	Toxin B PCR	62 (100)	15 (24.2)
Ellis et al., 1984 [27]	390	<2 years	390 (87.2)	Culture	191 (49.0)	13 (5.7)
Hjelt et al., 1987 [28]	153	6 months–7 years	153 (77.7)	Culture + presence of toxin	4 (2.6)	2 (50)
Kennedy et al., 1991 [41]	88	1 month–15 years	88 (100)	Culture + cytotoxicity	8 (9.1)	5 (62.5)
Kim et al., 2012 [17]	82	1.78–12.16 years	82 (100)	Culture + toxin PCR	69 (84.1)	3 (4.3)
Kim et al., 1989 [38]	335	0–18 years	335 (100)	Culture + toxin OR toxin presence only	36 (10.7)	34 (94.4)
Klein et al., 2006 [18]	372	0–28.6 months	688 (42.3)	Cytotoxicity	46 (66.9)	9 (19.6)
Kotloff et al., 1988 [19]	910	<2 years	910 (100)	Cytotoxicity	29 (3.2)	10 (34.5)
Langley et al., 2002 [20]	217	<2 years	217 (100)	Toxin immunoassay	39 (18.0)	1 (2.6)
Nivenius et al., 1987 [29]	157	<7 years	157 (100)	Culture + cytotoxicity	20 (12.7)	8 (40)
Niyogi et al., 1991 [39]	513	0–18 years	513 (100)	Culture	38 (7.4)	7 (18.4)
Oğuz et al., 2001 [30]	100	1 month–13 years	100 (100)	Culture + toxin immunoassay	24 (24.0)	6 (25)
Pai et al., 2012 [8]	75	0–16 years	Inclusion criteria	Toxin immunoassay + cytotoxicity	75 (100)	9 (12)
Pinto et al., 2003 [42]	210	3 months–7 years	210 (100)	Culture + cytotoxicity + toxin PCR	14 (6.7)	2 (14.3)
Rexach et al., 2006 [21]	977	1 month–19 years	977 (100)	Culture + toxin A/B PCR	326 (33.4)	84 (25.8)
Rosenfeldt et al., 2005 [31]	50	9–44 months	50 (51.0)	Culture	5 (10.0)	5 (100)
Sandora et al., 2011 [22]	1,891	1 month–18 years	1,891 (100)	Toxin presence	263 (13.9)	13 (4.9)
Shastri et al., 1998 [23]	357	0.2–16 years	357 (100)	Culture + toxin immunoassay	40 (11.2)	4 (10)
Thompson et al., 1983 [24]	208	0–18 years	208 (100)	Culture + toxin immunoassay	18 (8.7)	15 (83.3)
Tvede et al., 1990 [32]	337	10–14	337 (100)	Cytotoxicity	84 (24.9)	15 (17.9)
Uhnoo et al., 1986 [33]	616	0–14	616 (100)	Culture + cytotoxicity	86 (14.0)	39 (45.3)
Valentini et al., 2013 [34]	232	1 month–16 years	232 (100)	Culture + toxin B PCR	23 (9.9)	19 (82.6)
Vesikari et al., 1984 [35]	104	0–26 months	104 (100)	Culture + toxin immunoassay	28 (26.9)	8 (28.6)
Total	10,201		10,606		1,708 (16.1)	355 (20.8)

CDT = binary toxin

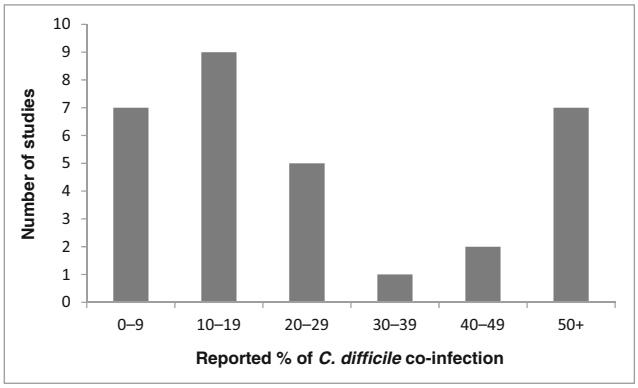

Fig. 1 Rate of co-infection with other gastrointestinal pathogens among *Clostridium difficile*-positive children in the 31 included studies

were found, details of the co-infecting pathogen were often not reported. Of the 355 children in whom a co-infection was found, in 133 patients (37.5 %), no specific organism was reported. Of the remaining 222 children, viruses accounted for most reported co-infections in *C. difficile*-positive children with diarrhoea (74 %, n=164), including rotavirus (59 %, n=97), adenovirus (20 %, n=32), norovirus (10 %, n=17), astrovirus (5 %, n=9), sapovirus (3 %, n=5) and others (2 %, n=4). Bacteria accounted for 53 cases (24 %), including *E. coli* (32 %, n=17), *Salmonella* spp. (21 %, n=11), *Campylobacter* spp. (21 %, n=11), *Yersinia* spp. (11 %, n=6) and others (15 %, n=8); co-infection with parasites was only reported in five cases (2 %).

Table 2 Number of reported gastrointestinal co-infections in *C. difficile*-positive patients by pathogen

Pathogen	Number of co-infection reports (%)
Viruses	164 (73.9)
Rotavirus	97 (43.7)
Adenovirus	32 (14.4)
Norovirus	17 (7.7)
Astrovirus	9 (4.1)
Sapovirus	5 (2.3)
Others[a]	4 (1.8)
Bacteria	53 (23.9)
E. coli	17 (7.7)
Enteropathogenic	8 (47.1)
Enterotoxigenic	3 (17.6)
Verocytotoxin-producing	4 (23.5)
O18	1 (5.9)
Not specified	1 (5.9)
Salmonella spp.	11 (5.0)
Campylobacter spp.	11 (5.0)
Yersinia spp.	6 (2.7)
Others[b]	8 (3.6)
Parasites	5 (2.3)
Blastocystis hominis	1 (0.45)
Entamoeba histolytica	2 (0.9)
Giardia spp.	2 (0.9)

[a] Calicivirus (n=2), coxsackievirus (n=1), enterovirus (n=1)

[b] *Bacillus cereus* (n=3), *Aeromonas* spp. (n=2), *Shigella* spp. (n=2), *Vibrio cholerae* (n=1)

Difference in disease severity with or without co-infection

Four studies assessed the impact of *C. difficile* co-infection with other gastrointestinal pathogens on clinical presentation [16, 26, 32, 34], but a clear correlation between a co-infecting organism and the presence of *C. difficile* on disease severity was not identified, and each study used different clinical markers of severity. The study by El Feghaly et al. [16] assessed the role of viral co-infections only in patients with a positive *C. difficile* test (aged 0–16 years), concluding that patient groups with (15/62; 24.2 %) and without (47/62; 75.8 %) viral co-infections were clinically indistinguishable, with a median time to resolution of diarrhoea on CDI therapy of 3 days, regardless of the viral co-infection status. Dulęba et al. [26] reported co-infection in 6/22 (27.3 %) children with severe CDI (defined by two or more of the following: fever ≥38.5 °C, white blood cell count ≥15,000/mm^3, elevated age-adjusted serum creatinine, albumin <2.5 g/dl) and 9/42 (21.4 %) children with non-severe CDI (age range 0–16 years), concluding that co-infection was not a significant risk factor for severe disease (p=0.83). There was no significant difference in the incidence of severe CDI among all age groups. Tvede et al. [32] reported that 17.9 % (15/84) of CDI cases had a co-infection with another pathogenic bacterial species, but excluded viral co-infection. However, symptoms and duration of diarrhoea did not differ from those with CDI alone. A recent study by Valentini et al. [34] suggested that *C. difficile* viral co-infections in children might influence the severity of clinical presentation. The study found a co-infection in 83.3 % (19/23) of *C. difficile*-positive patients (age range 0–16 years). Detection of *C. difficile* and rotaviruses were the most common (63 % of patients with any co-infection). Children with a co-infection in general had a more severe clinical presentation and had a higher probability of being severely dehydrated than those with mono-infection, independent of age and living in urban or rural areas. This analysis compared any co-infection with any mono-infection and was not specific for *C. difficile* co-infection.

Testing methods for *C. difficile*

Most studies in this analysis used the decision to test for *C. difficile* as an inclusion criterion. There are many different approaches that can be used in the laboratory to test for the

presence of *C. difficile*. However, the best standard laboratory test or combination of tests has not yet been fully established in children, although UK guidelines for adult disease incorporate a two-step approach of a screening test followed by a confirmatory test [43]. Commonly used tests include: (i) the detection of *C. difficile* products such as toxin A and/or B, glutamate dehydrogenase (GDH) or cell culture cytotoxicity; (ii) culture of toxigenic *C. difficile*; and (iii) polymerase chain reaction (PCR) amplification of 16S RNA, toxin genes or GDH. The studies included in this analysis utilised a variety of methodologies for the diagnosis of CDI (summarised in Table 1), including *C. difficile* culture, in vitro cell culture cytotoxicity, presence of toxin A/B based on PCR amplification and/or enzyme immunoassay, or a combination of these methods. Of the 31 studies in our analysis, 16 included testing for *C. difficile* and identification of free toxin when diagnosing CDI, ten tested for toxin only, while five of the studies defined CDI only as the presence of *C. difficile* culture (Table 1). In these five studies, the presence of toxigenic *C. difficile* was not confirmed, and to eliminate any potential bias in our analysis resulting from the inclusion of studies where non-toxigenic *C. difficile* may have been identified, we repeated the analysis after removing these studies. Of the remaining 8,882 patients from the 26 studies, 1,449 positive *C. difficile* tests were reported and co-infections were noted in 327 cases (22.6 %). This co-infection rate was similar to our observed pooled rate from all 31 studies.

Discussion

The findings of this review suggest that co-infection with *C. difficile* and other gastrointestinal pathogens is common in children with diarrhoea, with a pooled rate of reported co-infection of 20.7 % in *C. difficile*-positive children. However, although co-infection is an important factor in understanding and managing *C. difficile* in children, the literature is very limited. The majority of studies included in our initial literature search did not test for co-infections. In those that did, the panel of co-infections tested for varied considerably. Taking this into account, along with the fact that a causative organism is found in only 23.2–67 % of children with diarrhoea [44, 45], this review highlights an under-appreciation of co-infections in children, and the true rate could be substantially higher than the reported pooled rate. Unfortunately, the studies in our analysis often consisted of small cohorts (with a mean of 55 *C. difficile*-positive samples), were not stratified by age groups or risk factors, and outcomes such as survival, length of hospital stay and incidence of complications were not discussed in depth, preventing a more meaningful interpretation of the data. Consequently, larger multi-centre studies that systematically analyse co-infection would be beneficial to better understand the role of co-infection in the pathophysiology and prevalence of *C. difficile* in this patient population.

The impact of *C. difficile* in children with diarrhoea is often debated. Although severe CDI is reported to occur, most cases in this group tend to be asymptomatic and the current convention is to consider the presence of *C. difficile* in stools of patients under 2 years old as colonisation. Indeed, a recent expert panel meeting concluded that there is currently no accepted case definition of CDI in infants [5]. As a result, stool samples of children are not routinely sent for *C. difficile* testing [12] and guidelines suggest that an alternative aetiology should be considered in young paediatric patients with diarrhoea. We found that, when co-infections were tested for in children with a positive *C. difficile* test, a variety of pathogens were encountered (Table 2). This pattern is not specific to children with a positive *C. difficile* test and can be expected in any child with diarrhoea [44]. Unfortunately, only four studies systematically tested for the presence of viruses, bacteria and parasites, and the specific pathogen was not reported in over one-third of co-infections, which limits the reliability of the data and underlines the need for future studies.

It seems reasonable to assume that isolated co-infecting viruses, bacteria and parasites are a sufficient explanation for the presence of diarrhoea, in which case the detected *C. difficile* reflects an asymptomatic colonisation in infants and neonates. Alternatively, *C. difficile* in children could be the primary cause of diarrhoea, or contributing to an additive or synergistic clinical effect with another pathogen. The included studies were not conclusive regarding the impact of co-infection on CDI severity. The study by Valentini et al. noted a high rate of *C. difficile* co-infections [34] and more severe clinical presentation with higher probability of dehydration was observed in the group with any co-infection compared with those with a mono-infection. However, this and other studies did not observe a correlation between *C. difficile* co-infection and disease severity [16, 26, 32]. Due to the small sample sizes in the included studies, and, in particular, the small numbers of co-infected patients, statistical analysis of the data is problematic. The markers of disease severity also varied for each study, which makes any conclusion on the effects of co-infection unreliable. Further studies comprising larger patient cohorts and consistent clinical markers are necessary to identify any link between *C. difficile* co-infections and the severity of clinical presentation.

The current UK CDI management guidelines, suitable for adults and older children, recommend that testing for toxigenic *C. difficile* should involve a positive first test for *C. difficile* (e.g. GDH), followed by identification of free toxin in faeces to diagnose symptomatic CDI [46]. Of the studies in our analysis, only 16 included testing for *C. difficile* and toxin, while five could not differentiate toxigenic and non-toxigenic *C. difficile*. The differences in diagnostic approaches that we observed are unsurprising given that the dates of study

publication span more than three decades. Broad use of multiple-step algorithms for diagnosing CDI in future studies may allow for an improved understanding of whether *C. difficile* is the causative agent of diarrhoea in cases of co-infections. However, the sensitivity and specificity of the different tests in paediatric populations, and the issue of positive predictive value in infants where the colonisation rate is high, remain to be established.

Concluding remarks

Our analysis shows that, if a young child presents with diarrhoea and a stool sample is tested for *Clostridium difficile* and other gastrointestinal pathogens, co-infections are frequently found. However, deficiencies in the current literature preclude meaningful conclusions on the true rate of co-infection in this patient group and the age group where co-infection is clinically important. More robust future studies incorporating larger sample sizes, consistent case definitions and diagnostic testing for a broad panel of viral, bacterial and parasitic co-infections are necessary to improve our understanding and management of *C. difficile* in children.

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Conflict of interest DAB and JAK are employees of Astellas Pharma EMEA. DAE has received funding from Astellas Pharma EMEA for attendance at a scientific congress. SNF received funding from Astellas, Cubist and Actelion to attend an international meeting of experts in 2013 to discuss generic issues related to conducting trials of *C. difficile* in infants and has attended advisory boards for vaccine and antimicrobial manufacturers. All honoraria are paid to the university or hospital, with no personal payment of any kind.

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