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Case study

Clostridium difficile infections in young infants: Case presentations and literature review

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ABSTRACT

It has been assumed that symptomatic *Clostridium difficile* infections do not occur in young infants, as this specific group would lack specific *C. difficile* toxin receptors. As a consequence, it is often current practice not to test for *C. difficile* in neonates and young infants up to 2 years of age presenting with (bloody) diarrhea. The evidence to support this is, however, weak and largely based on small, poorly designed animal studies. We present two young infants with recurrent bloody diarrhea following antimicrobial therapy, positive testing for toxigenic *C. difficile* and successfully treated with metronidazole and vancomycin, and provide an overview of the literature on *C. difficile* infections in children under two years of age. Both our case histories and the literature search provide evidence for *C. difficile* infection as a potential cause of bloody diarrhea in neonates and young infants, in particular after previous treatment with antimicrobials.

Introduction

Bloody stools in neonates and small infants may be the presenting symptom of anal fissures, cow's milk allergy, juvenile polyps, congenital venous malformation and especially bacterial infection of the gastrointestinal tract [1]. *Clostridium difficile* infection is a relatively frequent cause of (bloody) diarrhea and pseudomembranous colitis in older children and adults, especially when its occurrence was preceded by antibiotic treatment, but is considered extremely rare in neonates and young infants. Percentage of bloody diarrhea (cases) in which *C. difficile* was the cause vary from 8%, 13% and 37.5% in patients below 1, 0–18 and 3–16 (median 2.1) years of age, respectively [2–4]. In adult patients, the number varies from 8.3%, 13.7% and 33% in patients younger than 65 years, older than 65 years and patients with a median age of 64 years (IQR 52–74), respectively [5,6]. C. difficile is an anaerobic, rod-shaped, spore-forming Gram-positive bacterium belonging to the phylum *Firmicutes*. *C. difficile*-associated diarrhea is caused by toxigenic strains, characterized by the production of toxins, including enterotoxin (*C. difficile* toxin A) and cytotoxin (*C. difficile* toxin B). In neonates, only a minority of *C. difficile* strains are considered pathogenic. Toxigenic *C. difficile* provokes mucosal inflammation and destruction of colonic epithelial cells, resulting in watery or bloody diarrhea, the latter case being a sign of pseudomembranous colitis [7]. Dysbiosis following the use of antibiotics is considered the most important underlying cause. This disturbance of the precarious balance of the commensal gut microbiota may induce overgrowth of toxigenic *C. difficile*, eventually leading to symptomatic infection [7–9].

Between 25 and 70% of neonates are colonized with *C. difficile* [7,8,10,11]. Within this specific age group, *C. difficile* may therefore be considered a component of the commensal microbiota. After two years colonization rates have decreased to 3–10%, remaining at that level throughout childhood and into adulthood [12]. In most, but not all [13], studies, nontoxigenic strains are reported to be more prevalent than toxigenic strains [11]. This was substantiated by the review of Jangi and Lamont, demonstrating that in pooled data of nine studies with a total of 928 healthy infants, 13% were carrier of toxigenic *C. difficile* strains and 17% of nontoxigenic strains [12]. The subgroup of toxigenic *C. difficile* strains consisted of different ribotypes with highly variable virulence phenotypes, including the hypervirulent 027 ribotype [14].

Although in adults and older children, toxigenic *C. difficile* is widely accepted as cause of (bloody) diarrhea, less consensus exists on the potential of *C. difficile* as causative pathogen of (bloody) diarrhea in neonates and young infants up to 2 years of age. We present two young infants with recurrent symptomatic infection with toxigenic *C. difficile*, who recovered completely following targeted antibiotic treatment.

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Case presentation

Case. A term, male neonate was born following uncomplicated pregnancy and delivery, birth weight 4860 g (> 2 SD). He was found to have hydrocephalus, spina bifida aperta and bilateral clubfeet. Postnatal diagnostic work-up, including MRI of the head and spine, revealed Arnold-Chiari malformation type 2, hydrocephalus and a myelomeningocele. Surgical closure of the spina bifida was performed at the age of four days. Up to twelve days of age the patient was treated with intravenous amoxicillin and subsequently discharged in good condition. As he needed intermittent catheterization because of bladder retention as a result of spina bifida, trimethoprim-sulfamethoxazole was prescribed as antibiotic prophylaxis. Nonetheless he experienced recurrent urinary tract infections, for which antibiotic courses were given with ceftriaxone, flucloxacillin and amoxicillin/clavulanic acid, respectively. At nine months of age the patient presented with frequent bloody diarrhea. Physical examination revealed no cause for the blood loss; length and weight were adequate. *C. difficile* toxin testing on feces by Enzym Immunoassay (EIA) was positive, and C. difficile was cultured from feces and from a biopsy of the sigmoid colon. Fecal culture for Salmonella, Shigella, Yersinia and Campylobacter (SSYC) species was negative. Tests for parasites, adeno- and rotavirus were also negative. Metronidazole prescription led to complete resolution of symptoms within a few days. EIA toxin testing of feces was negative at four weeks follow-up. Six weeks later, the bloody diarrhea recurred, accompanied by a body temperature of 39.5 °C. Fecal culture again was positive for toxigenic *C. difficile*. A second course of metronidazole was successful again, but two weeks later, symptoms and positive culture and EIA on feces had recurred. This time, oral vancomycin was prescribed, resulting in complete and definite resolution of the bloody stools and in negative results for *C. difficile* culture and EIA.

Case. An eighteen-month old boy presented with one week of bloody, foul-smelling diarrhea. Two months before she had been admitted because of acute tubular necrosis-induced renal insufficiency, treated with hemodialysis for two weeks as well as several antibiotics including third-generation cephalosporins and flucloxacillin. Fecal culture for SSYC was repeatedly negative, although from these cultures an Aeromonas species was isolated on one occasion. Real-time PCR for rota-, adeno-, and norovirus, and a triple feces test for parasites were negative. *C. difficile* testing on fecal samples was positive: *C. difficile* toxin gene PCR testing was positive, *C. difficile* could be cultured from feces, and toxins could be demonstrated in the feces by EAI. Abdominal ultrasound showed diffuse thickening of the colonic walls, suggesting colitis. After five days of unsuccessful treatment with oral metronidazole, vancomycin was prescribed. Bloody diarrhea resolved completely within a few days. At follow-up, *C. difficile* PCR, culture and toxin testing were negative.

Discussion

For considerable time it has been assumed that symptomatic infections do not occur in neonates and young infants, although colonization rate with *C. difficile* is very high in this specific age-group [10,15,16]. It has been suggested that the neonatal gut lacks *C. difficile* toxin receptor sites and has poorly developed cellular signaling pathways [17]. Consequently, testing for (toxigenic) *C. difficile* in neonates and infants below the age of two years has frequently been discouraged by clinicians and clinical microbiologists [18]. Policy statement of the American Academy of Pediatrics include the recommendation to limit testing for *C. difficile* in infants younger than 12 months of age presenting with diarrhea to those cases with severe motility disorders, Hirschsprung's disease or in outbreak situations. It was advised to look for alternative etiologies even in those infants with a positive test result for *C. difficile* [19].

However, reports discussing pathophysiological mechanisms on how young infants may be protected from developing symptomatic *C. difficile* infections have not been substantiated by robust scientific data and are merely hypothetical. A study that is often referred to was performed by Eglow et al., who found a decreased concentration of toxin A receptors in the ileum of newborn rabbits compared to adult rabbits, they predicted a comparable outcome in humans [20]. However, in *C. difficile* associated diarrhea it is commonly the colonic mucosa and not the small intestines which is affected. Furthermore, generalization of findings in animals towards humans is complex and may be questionable, in particular since observations in these studies were contradictory. For example, in contrast to the findings in newborn rabbits, Keel et al. described a high concentration of toxin receptors in the small and large intestines of neonatal pigs [21]. Claims on impossibility of development of symptomatic *C. difficile* infections in young infants due to the absence of intestinal toxin receptors in this specific population seem to be based on cross-references [22], ultimately referring to one small-scaled experimental study by Chang et al. [23]. This study described uptake of Clostridium toxin in a suspension containing adult colonic cells (n = 1 adult colonic sample), but no toxin uptake in a suspension containing fetal intestinal mucosal cells (n = 2 fetal intestines), obtained from two aborted fetuses in the second trimester [23]. It could be questioned whether the applied study design is sufficient to allow extrapolation of findings to the general (neonatal) population.

Alternative theories regarding the observation that young infants colonized with *C. difficile* do not suffer from symptoms direct to the potential positive effect of maternal antibodies transferred to the child by breastmilk, and the composition of the neonatal gut environment [11,12,24].

A review of the literature yielded at least eight studies including children below one year of age that strengthen the hypothesis that symptomatic *C. difficile* infections do occur in neonates and infants, with (bloody) diarrhea as the most frequently reported presenting symptom (Table 1) [2–4,13,25–28]. Majority of described cases had, similar to our presented cases, used antibiotics prior to onset of clinical symptoms. Despite these observations, the possible causality between toxigenic *C. difficile* and clinical symptoms remains subject of debate. The Bradford Hill criteria for causation states that causality is likely if there is a very specific population at a specific site and disease with no other likely explanation [29]. Hypothetically, the presence of toxigenic *C. difficile* could merely have been a biomarker of intestinal dysbiosis, while other non-cultivable pathogens, which also may react on antibiotic treatment, may have provoked the clinical symptoms. To establish a causative relationship between toxigenic *C. difficile* and presence of clinical symptoms, detailed insight in the pre- and post-treatment composition of the intestinal microbiota, preferably by means of molecular microbiota detection techniques, could shed light on this dilemma.

Alternatively, demonstration of the presence of intestinal *C. difficile* toxin receptors in neonates and young infants could scientifically support the hypothesis that symptomatic *C. difficile* infections do occur in this specific population, and should therefore be the focus of future studies.

Clostridium testing

Currently, several tests are available for the detection of toxigenic *C. difficile* in feces (Table 2), based on culture, enzyme immunoassay or polymerase chain reaction [30]. To distinguish between *C. difficile* infection and colonization, it is of importance to assess whether toxins or toxigenic *C. difficile* strains are present. This can be done by directly demonstrating the toxin or toxin genes in feces, or by demonstrating the toxigenic nature of cultured *C. difficile* isolates (toxigenic culture). To achieve an optimal diagnostic yield, a combination of testing modalities could be useful. When a diagnosis of *C. difficile* infection in a young

Table 1
Studies considering symptomatic *Clostridium difficile* infections in neonates and infants

	Patient group	Study design	Test	Test results	Treatment/response	Comment
Vesikari et al. 1984 [25]	Hospitalized children 0–2 years – 52 with diarrhea – 52 without diarrhea	Prospective case study	Toxin EIA	– 11/52 (21%) with diarrhea positive for *C. difficile*, 1/11 toxin positive – 17/52 (33%) without diarrhea positive for *C. difficile*, 5/17 toxin positive	NA	23/28 children tested positive for *C. difficile* had previous antibiotic treatment
Enad et al. 1997 [13]	87 NICU patients admitted during 4-month period and tested 2-weekly for *C. difficile* toxin A	Prospective cohort study	Toxin (A) EIA	45/87 (52%) toxin A positive	Blinding broken in 3 patients, all toxin A positive, treated with vancomycin: 1. Frequent foul stools 2. Frequent loose green stools, abdominal distention, dehydration 3. Abdominal distention, bloody stools (diagnosed with NEC)	No difference in previous antibiotics between toxin positive and toxin negative group
Ferreira et al. 2003 [26]	Hospitalized children 0–5 years – 90 with acute diarrhea – 91 controls	Retrospective case study	CCNA	Positive tests in – 2/44 patients 0–5 months – 2/22 patients 6–11 months – 1/24 patients 1–5 years 0/91 controls	NA	Some patients had previous antibiotic therapy
Morinvill et al. 2005 [2]	64 patients < 1 year with *C. difficile*-associated diarrhea (positive assay and GI symptoms): – 45 watery stools/diarrhea; – 5 bloody stools; – 14 no diarrhea	Retrospective case study	CCNA	– 64/64 positive tests – 7/64 (11%) rotavirus positive – 1 inflammatory bowel disease – 2 Hirschsprung disease	31/64 metronidazole 5/31 retreated for recurrent or persistent symptoms	46/64 were hospitalized within prior month; 54/64 (84%) had antibiotics within prior two months
Pai et al. 2012 [27]	75*C. difficile* toxin-positive children 2 days-14 years (median 2 years) Based on severity criteria: – 9 mild infection – 8 moderate infection – 57 severe infection – life threatening	Retrospective descriptive study	EIA followed by cytotoxin assay	– 75/75 positive tests – 3 adenovirus positive – 3 norovirus positive – 2 rotavirus positive – 1 enterovirus positive	30/75 metronidazole 3 treatment failures with solely metronidazole, escalation to vancomycin	55/75 (73%) had used antibiotics one month prior to infection
Khanna et al. 2013 [28]	92 children 1 month-17 years (median 2.3 years; 16 children < 1 year) with toxinogenic *C.difficile* diarrhea	Population based cohort study	Toxin EIA or PCR	92/92 positive tests	92 metronidazole (13 failures); 7 vancomycin, 2 rifaximin (no failures) Overall recurrence rate 20%	72/92 (78%) had antibiotics prior to infection
Duleba et al. 2014 [4]	64 children 3 months-16 years (median 2.1 years; 9 children < 1 year) with *C. difficile* diarrhea	Retrospective case study	PCR	– 64/64 positive test – 8 rotavirus positive – 2 *Salmonella* positive – 2 *Yersinia* positive – 1 adenovirus positive – 1 norovirus positive – 1 *Campylobacter* positive	36/64 metronidazole, 8/64 vancomycin and metronidazole, 6/64 vancomycin, 1/64 rifaximin (*Salmonella* coinfection), 13/64 no treatment Good response in 49/51 Recurrence in 3 patients	61/64 (95%) had previous antibiotics
Samady et al. 2014 [3]	– 134 patients 0–18 years (28 children < 1 year) with *C. difficile* diarrhea – 274 controls (58 < 1 year) with *C. difficile*-negative diarrhea	Retrospective case-control study	Toxin EIA	– 134/134 positive test – 0/274 controls positive test	NA	88/134 (66%) *C. difficile* patients and 55/274 (20%) controls had previous antibiotics

Table 2
Laboratory tests to diagnose *Clostridium difficile*.

Test	Target	Description
EIA for *C. difficile*	Glutamate dehydrogenase	Detection of *C. difficile* glutamate dehydrogenase in feces. Short turnaround time, but low specificity. No distinction between toxin producing and non-toxin producing *C. difficile*.
EIA for toxin	Toxin A and B	Immunoassay directed at toxin A and B. Short turnaround time, limited sensitivity.
Toxigenic culture	Toxin producing *C.difficile*	Selective culture of *C. difficile* with immunoassay detection of toxins. Positive when *C. difficile* is cultured and direct toxin testing of stool is positive *or* toxin testing of *C. difficile* isolates is positive. Relatively long turn-around time, but sensitive and yields isolates for epidemiologic purposes.
CCNA	Toxin B	Induction of cytopathic effect by fecal *C. difficile* toxin B. Relatively long turnaround time, expensive and laborious. Historically seen as the gold standard.
Real-time PCR	Toxin A and B genes	Based on amplification of the genes encoding toxins A and B. Rapid and highly sensitive. No discrimination between colonization and overgrowth of *C. difficile*.

infant is established, initial treatment with metronidazole orally (30 mg/kg/day for 10 days) is recommended [19,31]. In case of therapeutic failure or relapse, vancomycin orally (40 mg/kg/day for 2 weeks or tapering) can be prescribed. A prolonged treatment course with tapering may be chosen in selected cases [19,31]. Although fecal transplantation in recurrent *C. difficile* infections in adults has been proven superior as therapy compared to prolonged antibiotic treatment, studies on efficacy of this relatively novel therapeutic approach in children with recurrent *C. difficile* infections are currently lacking [32].

Conclusion

Based on the assumption that symptomatic *C. difficile* infections do not occur in children below two years of age, current policy of several microbial laboratories is not to test for these infections in this specific population. The evidence to support this regime is weak and largely based on small scaled, poorly designed animal studies. Based on a literature search and illustrated by two case reports, we recommend to include symptomatic *C. difficile* infections in the differential diagnosis of (bloody) diarrhea in neonates and infants, especially following (prolonged) use of antibiotics. Demonstration of toxin receptors in this population should be the focus of future studies to scientifically support this recommendation.

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